In Vitro Cell. Dev. Biol.—Animal 38:59–61, February 2002

1071-2690/01 $10.00+0.00

Letter to the Editor

DELIVERY AND STORAGE OF SINGLE EMBRYOS, SPERM, OR CELLS IN MICROGLASS CAPILLARIES

Dear Editor:

In general, a large number of cells can be placed in glass or plastic tubes for delivery or storage (or both). In these cases, the loss of some cells does not affect further experimental procedures or analyses. However, in some cases, only a few cells, such as gametes and embryos, can be obtained from humans or rare animals for further analysis. For example, it has been reported that single cells can be analyzed by polymerase chain reaction (Li et al., 1988; Monk and Holding, 1990; Zhang et al., 1992; Dietmaier et al., 1999). In human-assisted reproduction, offspring can be produced by intracytoplasmic sperm injection (ICSI), and normal fetuses may be obtained by microinsemination with frozen–thawed round spermatids collected from obstructive azoospermic males (Abuzeid et al., 1997). Offspring of mice may be obtained by microinsemination with frozen–thawed spermatids of azoospermic males and spermatozoa stored in alcohol (Tanemura et al., 1997; Tateno et al., 1998). Normal offspring may also be obtained from mouse oocytes injected with spermatozoa after cryopreserving with or without cryoprotectants or by using freeze-dried spermatozoa (Wakayama et al., 1998; Wakayama and Yanagimachi, 1998). Indeed, for further analysis or research, reliable and safe delivery of one cell or embryo from one location to another is often necessary. In this study the two separated halves of zona pellucida, the embryos reconstructed by nuclear transfer, and the sperm and somatic cells at different densities were stored in glass capillaries and delivered more than 1000 km by mail within 1 wk.

Glass capillaries with 1.0- or 1.3-mm outer diameter (O.D.) and 0.9-mm inner diameter (I.D.) were employed. Using an alcohol burner, a tip of about 30 mm in length and having a 100-μm O.D. is pulled on to one end of the glass capillary; the tip and the opposite end (base) of the glass pipette are then fire-polished. A silicone tube with 1.0-mm I.D. is connected to the base of the pulled capillary, which in turn is connected to another silicone tube with 2.0-mm I.D. to form a mouth-controlled pipette (Wang et al., 1999).

Using a stereomicroscope or inverted microscope, mineral oil and an air bubble are aspirated into the tip of the capillary. Then, a column of medium containing the cell, the embryo, or the cellular structure to be stored or delivered is aspirated into the capillary, followed by mineral oil. The last oil droplet is at least 5 mm from the tip of the capillary tube. The tip of the capillary is checked using an inverted microscope to ensure that the cell or the embryo is contained within the medium. The arrangement in the filled capillary is shown in Fig. 1. The two ends of the capillary are then flame-sealed. Coded marks are then made on the neck or the mid-region (or on both) of the capillary for identification. In total, six marks can be made with a marking pen along the surface of the capillary, as shown in Fig. 1, and can reliably identify up to 20 individual capillaries when employed in the manner depicted. For delivery to other laboratories, the capillaries are placed into a hard-wall container, such as a plastic or metal tube or box whose space is filled with cotton or soft material to fix the position of the capillaries.

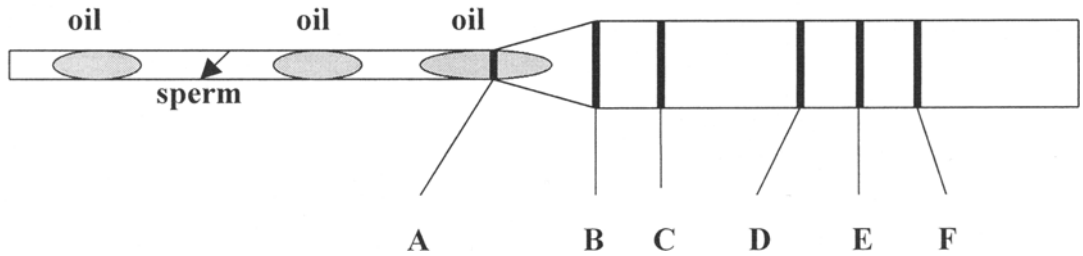

FIG. 1. The package and marking of capillaries for identification.

Coding the microglass capillaries can be done by drawing a series of lines or dots with a marking pen along the capillary surface. The locations of the marks are as follows: mark *A* (neck), mark *B* (shoulder), mark *C* (body), marks *D*, *E*, and *F* (middle to base).

Code Number:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Position of mark(s) on capillary:	A	B	C	A B	A B C	A D	B D	C D	A B D	A B C D	A D E	B D E	C D E	A B D E	A B C D E	A B E F	B D E F	C D E F	A B D E F	A B C D E F

In this system up to 20 capillary tubes can be sent or stored together for identification at a later period.

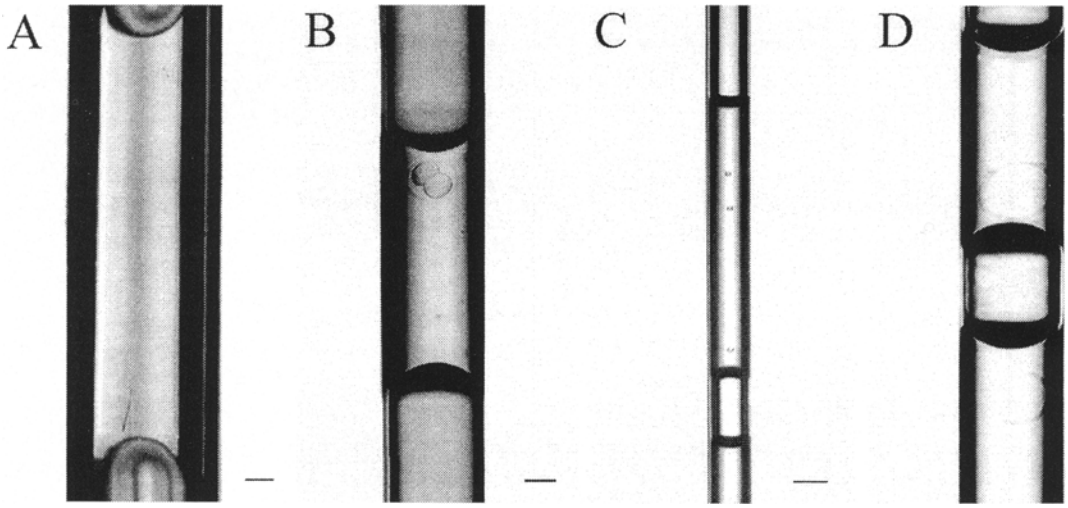

FIG. 2. Arrangement and position of sperm, embryos, or cells and zonae pellucidae in the microglass capillary. (*A*) A sperm, (*B*) a two-cell embryo, (*C*) three cells, and (*D*) two zonae pellucidae. Bar, 50 μm.

We have delivered 20 capillaries of panda somatic cells, 25 capillaries of individual embryos from the two-cell stage to the blastocyst stage, and 22 capillaries of couples of separated halves of zona pellucida. After delivery, both ends of the capillary were removed by a sand gear or a diamond leaf. All the cells, separated zonae pellucidae, and embryos were recovered, and none were lost. The arrangement and position of single sperm, embryos, zonae pellucidae, or cells in capillary tubes are shown in Fig. 2*A–D*.

Freezing two-cell stage embryos of Kunming strain mouse (KM × KM) in microglass capillaries, as described earlier, was carried out using the method of Nakagata (1989). In these instances, or where individual cells are to be frozen, the ends of the capillaries are not sealed. Embryos were recovered from liquid nitrogen 1 to 5 d after storage and cultured in M16 + ET medium (Wang et al., 2000) for 72 h. Using this method, 24 out of the 30 embryos (80%) developed into the blastocyst stage. The percentage of blastocysts was lower than that of embryos without freezing but similar to those from other freezing methods (Wang et al., in prep.).

Because of the transparency of glass capillaries, cells, embryos, and zonae pellucidae can be clearly observed during filling and emptying of the capillaries. The pulled tip of the capillary is flexible and not easily breakable. In addition, no contamination occurred during this entire operation and delivery because the capillaries were sterilized at the time at which they were pulled. Compared with the plastic straws for storage of embryos in liquid nitrogen (Vajta et al., 1998), the glass capillary is more transparent and can be pulled thinner. It has been reported that sperm can be stored with zona pellucida, but micromanipulation is needed to recover the sperm, and this method has the possibility of introducing proteins attached to the zona pellucida (Cohen et al., 1997) to preparations when the sperm are transferred. Use of glass capillaries, as described here, can avoid such problems. In conclusion, storage or delivery (or both) of one or more cells, embryos, or sperm in the tip of a capillary is simple, convenient, safe, and economical.

ACKNOWLEDGMENTS

We are indebted to Ms. Xiang-Fen Song for her excellent technical assistance and to Dr. Sanjeev Chaubal and Dr. Flank J. Longo, College of Medicine, University of Iowa, for their helpful comments and critical reading of this letter. The present study was supported by grants from the Climbing Project in China (97021109-2) and the China National Natural Scientific Foundation (39360028).

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(Received 28 December 2000)