nary experiments indicate that the presence of the zona pellucida is required for post-thaw embryo development. Embedding in agar may improve the developmental capacity of zona pellucida free embryos. The rate of development of bisected embryos, however, was low and not increased by the presence of agar.

172. *The Kharkov Technology of Cattle Embryo Cryopreservation.* F. I. OSTASHKO, N. D. BEZUGLY, V. V. PESOTSKY, N. A. GORDIENKO, AND E. G. VOLKOVA (Research Institute of Animal Breeding of the Forest-Steppe and Woodlands, Kharkov, USSR).

Our technology of cattle embryo cryopreservation, which we call Kharkov, consists of the following stages: (a) one-step embryo introduction by a 1.2 *M* glycerol (Gl) solution in 20% fetal serum (FS) in phosphate-buffered saline (PBS) for 7 min; (b) embryo preservation at 0°C if more than 2 h remain before the start of freezing; (c) freezing at a constantly reducing rate (from 10°C to 0°C/min) for 60 min, exposure for 30 min at −30°C, and transfer into liquid nitrogen; the composition and disposition order of the media in the freezing container provide spontaneous ice seeding at temperatures not below −6°C; (d) thawing by plunging the container into a room temperature water bath; (e) immediate Gl removal from the embryo for 5 min in the container by mixing the freezing medium with a 0.75 *M* sucrose solution in 20% FS in PBS; (f) embryo evaluation and transplantation in the freezing container. The parameters of the main technological stages were determined by physical and mathematical modeling on the basis of embryo properties and embryo cryoresistance. This technology provides embryo survival not less than 90% and pregnancy not less than 40%.

SESSION XVIII—SPECIAL TOPICS

173. *Cryopreservation of Rumen Protozoa.* F. GYULAI, A. MARCIN, AND M. SOROKOVÁ (Slovak Academy of Sciences, Institute of Animal Physiology, Košice, Czechoslovakia).

A simple technique for the cryopreservation of the rumen ciliate *Entodinium simplex* is described. After anaerobic equilibration at 312.2°K with cryoprotectant the protozoal suspension was transferred into Eppendorf polyethylene tubes. The tubes with protozoal samples were placed into a cooling vessel consisting of an internal container insulated by commercial granulated building material (Perlit). The bottom of the cooling vessel was placed 20 cm above the level of liquid nitrogen (LN_2) in a 30-liter Dewar flask during cooling of the protozoal samples from 296.5° to 173.2°K. When the tubes were at 173.2°K they were immediately immersed in LN_2. After 2 months storage in LN_2 the protozoa samples were warmed in a water bath at 313.2°K and the presence of motile protozoa was evaluated microscopically. The growth ability of the cryopreserved protozoa was confirmed by cultivation. *E. simplex* survival of about 9% was observed by using 30 min equilibration of protozoa with 3% DMSO as cryoprotectant, a concentration of 60,000 protozoa/ml, and a cooling rate obtained with the described technique. The possibility of cryopreservation of other species of rumen protozoa is discussed.

174. *Loss of Plasmid Expression in Yeasts following Freezing and Thawing.* G. J. MORRIS AND B. PEARSON (Cell Systems, Ltd., Cambridge Science Park, Cambridge, United Kingdom).

The effects of freezing and thawing on the expression of a range of genetically engineered plasmids in yeast cells were examined. Following rapid rates of cooling there was a decrease in the percentage of the population which expressed plasmid. This was not a selection of preexisting plasmid free cells; analysis of DNA from rapidly frozen cells demonstrated that partial deletions of plasmid DNA had occurred. These results are discussed in terms of the physical stresses the cells are exposed to during freezing and in relation to the development of cryopreservation methods for genetically engineered yeasts.

175. *Cold-Induced Influences on Insect Metamorphosis: Developmental and Seasonal Considerations.* K. L. HORWATH (Center for Cryobiological Research, State University of New York, Binghamton, New York).

Perhaps the most spectacular postembryonic developmental event in insects is metamorphosis. Its timing is usually synchronized by environmental triggers to ensure that growth and reproduction occur at the most optimal times. During periods of unfavorable environmental conditions cold hardiness and diapause become important. These developmental events are also specifically timed. This paper addresses the hypothesis that how insects become programmed for a cold hardy (or diapause) state on a seasonal basis may be related to how they become committed to a particular differentiated state during development, and also explores the influence of environmental stimuli (i.e., temperature) in this regard. Fifth instar larvae of the tobacco hornworm *Manduca sexta,* were low temperature (0, 10, 15°C) pulsed for various periods of time prior to and during the pupal commitment switch of the dermal glands (18–36 hr after ecdysis). The impact on larval–pupal transformation was assessed by SDS–PAGE of stage-specific protein products. Results suggest cold-induced alterations of the temporal patterning of the metamorphic hormones directing pupal commitment, and additional physiological changes imparted by low