

hours. Performing Testicular Sperm Extraction (TESE) 2 days prior to OPU appears to increase the pregnancy rates significantly.

## ART: CRYOPRESERVATION

Wednesday, October 25, 2000

### P-376

**Cryopreservation of Mouse and Human Embryos Using Ethylene Glycol (EG) in Controlled Slow Freezing.** H. J. Chi, M. Y. Kim, J. Y. Ju, J. Y. Kim, J. J. Koo, S. S. Chang. IVF & Laparoscopy Center, Hanna Women's Clinic, Seoul, Korea.

**Objective:** Ethylene glycol has been successfully used as a cryopreservative for vitrification of mammalian embryos including human. Due to its lower molecular weight (62.07 g/mol) compared to other cryopreservatives, it is possible to get a better result if EG is used for cryopreservation of mammalian embryos in slow freezing, instead of propandiol (PROH). This study was carried out: (1) to compare permeability and toxicity of EG with those of PROH using mouse embryos, (2) to investigate the efficiency of EG in relation to embryo cleavage stage, and (3) to determine whether pregnancy outcome is improved when human embryos are frozen by using EG instead of PROH.

**Design:** A prospective study on cryopreservation of human embryos using EG in slow freezing was performed, from Jan. 1999 to Dec. 1999. As a control group, the retrospective data of the cryopreserved human embryos using PROH, from Jan. 1998 to Dec. 1998, were used.

**Materials and Methods:** Mouse embryos were stepwise exposed to 1.5M PROH or EG with 0.2M sucrose and then some of the embryos were cryopreserved and the others were stepwise washed and cultured. Extra human embryos after embryo transfer were also cryopreserved using 1.5M PROH or EG with 0.2M sucrose in controlled slow freezing.

**Results:** When 2-cell mouse embryos were exposed to EG (n=352) or PROH (n=358), there was a significant difference in the percentages of embryos developed to expanded blastocyst between EG (64.4%, P<.05) and PROH groups (53.8%). The mouse embryos exposed to PROH showed a cytoplasmic shrinkage, but the embryos exposed to EG did not. Two-, 4-, and 8-cell embryos were frozen/thawed with PROH or EG, the overall survival and developmental rates of the embryos in EG group were 69.1 and 33.1%, respectively, which were significantly higher than the rates (9.1 and 3.0%, P<.05) of the embryos in PROH group. The survival rates of 2-cell mouse embryos in both EG and PROH groups (76.1 and 15%, P<.05) were significantly higher than the rates of 4- (66.7 and 3.7%) or 8-cell embryos (64.7 and 8.7%). However the developmental rate of 8-cell embryos in EG and PROH groups (63.8 and 9%, P<.05) were significantly higher than the rates of 2- (6.4 and 0%) and 4-cell embryos (29.3 and 0%). The survival rate of human embryos frozen/thawed with EG (72.2%, P<.05) was significantly higher than the rate of embryos frozen/thawed with PROH (54.7%). Although the pregnancy rate of embryos in EG group (31.4%, 11/35 cycles) was higher than the rate of embryos in PROH group (20.5%, 7/34 cycles), but there was no statistical significance in the difference of the rates.

**Conclusions:** We observed that lower toxicity and higher permeability of EG compared to PROH. Cryopreservation of human embryos using EG increased the survival and pregnancy rates compared to the use of PROH. Therefore EG may be a better substitute for PROH to cryopreserve human embryos in slow freezing protocol.

### P-377

**The Effect of Cryopreservation on the Spontaneous Acrosome Reaction of Human Spermatozoa.** A. Abderazek, I. Fahmy, R. T. Mansour, G. I. Serour, C. A. Rhodes, M. A. Aboulghar. The Egyptian IVF-ET Centre, Cairo 11431, Egypt.

**Objectives:** Cryopreserved spermatozoa are widely used in assisted reproduction programs. While processing and incubation increase the rate of spontaneous acrosome reaction in human spermatozoa, little is known about the effect of cryopreservation. This study was conducted to assess the effect of cryopreservation on the spontaneous acrosome reaction of human spermatozoa. To the best of our knowledge, this is the first study to include a period of incubation before assessing the rate of acrosome reaction.

**Design:** A prospective comparative study of thirty normal human ejaculates according to WHO guidelines.

**Materials and Methods:** Each ejaculate was divided into two equal portions. The fresh portion was processed using tissue culture media and incubated for three to five hours. The second portion was cryopreserved for seven days, after which it was thawed, processed in the same manner and incubated for the same period of time. The hypo-osmotic swelling test was then applied to both portions for identification of living spermatozoa. Evaluation of the spontaneous acrosome reaction was done after staining with pisum sativum agglutinin (FITC-PSA), using the fluorescent microscope.

**Results:** The percentage of living spermatozoa with spontaneously reacted acrosome in the fresh and cryo-thawed samples were  $21.7 \pm 3.9\%$  and  $28.8 \pm 3.2\%$  respectively (mean  $\pm$  standard deviation). This difference was found to be statistically significant (p<0.001).

**Conclusion:** Cryopreservation increases the incidence of spontaneous acrosome reaction in ejaculated sperm after a period of incubation.

### P-378

**Factors Relating to a Successful Cryopreserved Blastocyst Transfer Program.** B. Behr, J. Gebhardt, J. Lyon, A. A. Milki. Department of Gynecology/Obstetrics, Stanford University Medical Center, Stanford, CA.

**Objectives:** Blastocyst embryo transfer has become an increasingly popular tool for selection of viable embryos prior to ET or cryopreservation. Much debate still surrounds the efficacy of blastocyst cryopreservation. The objective of this study is to review our experience with frozen blastocyst transfer with regard to (1) day frozen, (2) status of the blastocyst post thaw and (3) pregnancy outcome.

**Design:** A retrospective review of non-donor patient cycles undergoing frozen blastocyst transfer at our center since January 1999. Patients including all ages were managed and transferred by the same physician.

**Materials and Methods:** All blastocysts were frozen utilizing the Menezo two-step protocol with mHTF and 20% SSS (Irvine Scientific) as the base medium and the Testart slow cool cryopreservation program. Blastocysts were thawed according to the patient's cycle using the two-step thaw protocol using the same base medium. The time from thawing to transfer was a ranged from 2.5 hours to 7 hours. Thawed blastocysts were incubated in Blastocyst culture media supplemented with 10% SSS (Irvine Scientific) prior to FET. Patients were prepared for FET on a natural cycle with an hCG shot when the leading follicle reached 17mm in diameter and luteal support with micronized vaginal progesterone 200mg BID starting 4 days after hCG injection.

All transfers were performed using the tefcat catheter (Cook Urological) 7 days after hCG.

**Results:** A total of 35 thaw cycles were performed and all had at least one surviving blastocyst for transfer. Of the 35 cycles, 7 were in patients over age 40. The average number of blastocysts transferred was 2.6 per patient. Thirteen clinical pregnancies resulted (37%) of which 11 are ongoing or delivered (31%). There were 14 transfers from blastocysts frozen on D5 which resulted in 6 clinical and 5 ongoing pregnancies (36%) compared to 21 transfers from blastocysts frozen on D6 which resulted in 7 clinical and 6 ongoing pregnancies (29%). In the over 40 group, there were 3 clinical and 2 ongoing pregnancies out of 7 transfers. The implantation rate was 18%. In all of the positive FET cycles, at least one fully expanded blastocyst was transferred. Only 2 patients with fully expanded blastocysts did not conceive. Two patients had hatching blastocysts transferred and both conceived.

**Conclusion:** Integrity of the thawed blastocysts transferred seems to be the determining factor for positive pregnancy outcome, namely the presence of at least one fully expanded blastocyst for ET. Day 6 blastocysts still have a good pregnancy potential when transferred into a D5 endometrium. The cryopreservation, thaw and ET of hatching blastocysts does not appear to be detrimental. With this preliminary experience, it appears that blastocyst cryopreservation is a viable option for all patient age groups.

### P-379

**Plasma Membrane Translocation of Phosphatidylserine and Determination of Reactive Oxygen Species: A Study of Their Association With the Cryopreservation-Thawing Survival of Fractionated Human Spermatozoa.** <sup>1</sup>M. Morshedi, <sup>2</sup>N. Duru, <sup>1</sup>S. Oehninger. <sup>1</sup>The Jones Institute for Reproductive Medicine, Department of Obstetrics/Gynecology, Eastern Virginia Medical School, Norfolk, VA, USA and <sup>2</sup>Department of Obstetrics/Gynecology, GATA School of Medicine, Ankara, Turkey.