seem to be equally efficient in maintaining oocyte survival and parthenogenetic development. However, the Reprogen protocol is a more simplified method requiring fewer steps and equipment. Furthermore, the Reprogen protocol allows for the storage of more oocytes in a single cryostraw and can therefore be considered as an economically better option for oocyte vitrification.

Supported by: Vitrification and thawing solutions were provided at gratis from both manufacturers.

P-175

EFFECT OF N-ACETYL GLUCOSAMINE AND B GLYCEROPHOSPHATE SUPPLEMENTATION IN CRYOPRESERVATION MEDIA ON POST THAW SPERM FUNCTIONAL CHARACTERISTICS. A. C. Varghese, S. Kundu, D. Mukhopadhya, J. Bhattacharya, A. K. Bhattacharyya, A. Agarwal. Univ of Calcutta, Calcutta, India; IVF & Infertility Research Center, Calcutta, India; Cleveland Clinic, Cleveland, OH.

OBJECTIVE: The cryosurvival rates of human spermatozoa remain poor despite of modern cryopreservation methods for human semen. This is due to factors such as membrane damage and sub lethal damage at chromatin level. The goal of the present study was to investigate the supplementation of N-acetyl-D-glucosamine (NAG), a major structural element in cell walls and coats, and β-glycerophosphate (β-GP) in cryoprotective agent (CPA) on post thaw sperm characteristics.

DESIGN: Prospective study.

MATERIALS AND METHODS: Semen samples (n = 19) were frozen with glycerol based CPA with a final concentration of 10mM N-acetyl-D-glucosamine in semen CPA mixture (1:1) and a second group of samples (n = 23) frozen with β-glycerophosphate supplemented CPA at a final concentration of 2.5mM. In both groups one aliquot each served as control without any additive in CPA. Following post thaw, % motility, % forward progressive motility (FPM) were assessed using Makler couting chamber. Percent normal morphology, % head defects were estimated by PAP staining technique. DNA integrity (%) was analyzed with acridine orange fluorescence, and lipid peroxidation (LPO) status measured by estimation of malonaldehyde formation by spectrophotometry.

RESULTS: There was a significant improvement in % motility (34.5 $\pm$ 14.8 vs. 27.1 $\pm$ 12.4, p = 0.023), % FPM (26.4 $\pm$ 4.4 vs. 18.9 $\pm$ 12.5, p = 0.019), % normal DNA integrity (74.0 $\pm$ 16.7 vs. 58.4$\pm$ 22.2, p = 0.003) in the NAG group vs.control. However, there was no improvement in % normal morphology or any reduction in LPO status compared to the control. Samples frozen with β-GP showed no significant improvement in motility patterns, LPO or DNA integrity. While a significant improvement in % morphologically normal forms was noted in this group (52.7 $\pm$ 4.3 vs. 44.4 $\pm$8.5, p = 0.020).

CONCLUSION: N-acetyl-D-glucosamine could protect human sperm against damage during freeze-thaw process. This may be due to its antioxidant property. Though the exact mechanism of action of β-glycerophosphate is not known, it may be hypothesized that this molecule may confer membrane stabilization and provide phosphate group for energy synthesis. Though inhibition of lipid peroxidation by β-GP has been reported earlier in other cell systems, no change was observed in the present study on LPO status of spermatozoa frozen with or without this additive.

Supported by: None.

P-176

A COMPUTER ASSISTED SPERM MOTILITY BASED STUDY ON RAPID VS. SLOW THAWING TEMPERATURES ON HUMAN SPERM MOTILITY PARAMETERS. A. K. Bhattacharyya, A. C. Varghese, S. M. Bhattacharya, P. Ray, J. Bhattacharya, A. Agarwal. Univ of Calcutta, Calcutta, India; Vivekananda Institute of Medical Sciences, Calcutta, India; Ashok Laboratory, Calcutta, India; IVF & Infertility Research Center, Calcutta, India; Cleveland Clinic, Cleveland, OH.

OBJECTIVE: In animal studies it has been shown that very rapid thawing at 65°C for 7.5 seconds provided optimal preservation of sperm motility and acrosome integrity. Cellular damage may be caused during the thawing process as the ice melts or re-crystallizes. Slow thawing is most likely to induce injury, as it allows time for consolidation of microscopic ice crystals into larger forms, which are known to be damaging. Some workers have reported that thawing human sperm at 37°C for 10 min was superior to room temperature for 30 min. The purpose of this study was to compare the difference in sperm characteristics in post thaw semen samples undergoing slow (37°C) and rapid thaw (55°C) procedure.

DESIGN: Prospective study.

MATERIALS AND METHODS: The study includes 15 semen samples of men attending the Andrology Laboratory. Each semen sample after liquefaction was divided into two equal parts and frozen using glycerol-based cryoprotectant (Sperm Freeze, Ferti Cult™, Belgium). For the comparative study on thawing temperature and survivability, aliquots of the same samples were thawed at 37°C (10 minutes) and 55°C (3 minutes). Post-processing samples were analyzed using CASA (Hamilton-Thorn, CEROS) for motility parameters.

RESULTS: Semen samples thawed at 55°C for 3 minute had higher % motility (12.0 $\pm$ 10.8 vs. 19.8 $\pm$ 12.7, P-value = 0.010) and % rapid forms (9.8 $\pm$ 9.8 vs. 15.3 $\pm$ 13.1, P = 0.030) compared to the sample thawed at 37°C. There were no significant differences in other CASA velocity parameters like VAP, VSL, VCL, ALH, BCF and linearity between the two groups.

CONCLUSION: Our results indicate that a short exposure to higher temperature may be beneficial to recover higher percentage of motile sperms from cryopreserved human semen. This may be due to a shortening of the re-crystallization time, an event that can have considerable impact on cell damage due to the physical and osmotic forces exerted at this stage.

Supported by: None.

P-177

AGONIST OR ANTAGONIST GNRH PROTOCOLS PROMOTES-THE SAME OUTCOMES OF SUBSEQUENT FROZEN-THAWED CYCLES. L. G. Maldonado, W. C. Busato, T. C. Bonetti, F. F. Pasqualotto, A. Iaconelli Jr., E. Borges Jr. Fertility - Assisted Fertilization Center, Sao Paulo, Brazil; Univ of Caxias do Sul, Caxias do Sul, Brazil.

OBJECTIVE: Controlled ovarian stimulation (COS) is a critical step in *in vitro* fertilization (IVF) therapy. Although excellent outcome have been obtained with either GnRH agonist or GnRH antagonist protocols, speculations about adverse effect of antagonists on embryo or endometrium support the differences in results using those protocols. Although the well-established frozen-thawed embryo transfer protocol may increase the cumulative pregnancy rates of IVF, little data is available about the direct effects of GnRH in subsequent cycles using freeze-thawed embryos. The purpose of this study was to evaluate the cycles outcomes with freeze-thawed embryos transferred, in patients submitted to GnRH agonist or GnRH antagonist protocols, during previous ICSI cycles.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: This study included 204 frozen-thawed embryos cycles between 2003 and 2005 in an assisted human reproduction private unit in Brazil. Two groups were established in according to COS protocol: *agonist*: long protocol with GnRH agonist (leuprolide 500 μg), or *antagonist*: GnRH antagonist (cetrorelix 250 mg). In both groups were used recombinant-FSH (Gonal-F®, Serono) in step-down protocol, and embryos were cryopreserved using a slow-freezing protocol with 1.5M dimethylsulphoxide as the cryoprotectant. Frozen-thawed cycles were evaluated regards to embryo survive, implantation and pregnancy rates. Statistical analysis was performed using Student t and $\chi 2$ tests as appropriate. A P-value $<$ 0.05 was considered statistically significant.

RESULTS: Cycles in which embryos were freeze, 33.3% used GnRH agonist, 27.2% used GnRH antagonist (p=0.150), and in 39.5% the embryos were not thawed. The age of women included in the study were similar between groups (agonist: 35.1 $\pm$ 6.5 *versus* antagonist: 34.7 $\pm$ 6.2; p=0.682). In spite of the period of embryo cryopreservation had been higher on agonist (7.4 $\pm$ 12.4 months) than antagonist group (4.7 $\pm$ 6.4 months, p=0.044), the number of embryos thawed by group were similar (agonist: 5.5 $\pm$ 2.5 *versus* antagonist-group: 5.5 $\pm$ 2.7; p=0.918), with an embryo survival rate of 60.3% and 86.0%, respectively (p=0.233). When it was evaluated the number of viable and intact embryos, the agonist and antagonist GnRH protocols were also similar, respectively (3.2 $\pm$ 1.9 *versus* 3.2 $\pm$ 2.1; p=0.898, and 1.3 $\pm$ 1.5 *versus* 1.4 $\pm$ 1.9; p=0.759). The number of embryos transferred by group were comparable (agonist: 2.7 $\pm$ 1.4 *versus* antagonist: 2.9 $\pm$ 1.3; p=0.407),

and the same implantation (agonist: 12.1% *versus* antagonist: 13.3%; $p=0.774$), pregnancy (agonist: 26.8% *versus* antagonist: 28.9%; $p=0.787$) and miscarriage (agonist: 18.9% *versus* antagonist: 38.5%; $p=0.169$) rates were obtained.

CONCLUSION: Embryo cryopreservation has been successfully carried out by several investigators resulting satisfactory ongoing pregnancy rates. In addition, the results of the present study suggest that transfer of those embryos originating from agonist or antagonist GnRH protocols showed similar outcomes in embryo survival, implantation, pregnancy and miscarriage rates.

Supported by: None.

P-178

PREGNANCY FOLLOWING FROZEN/THAWED TRANSFER OF DAY 7 BLASTOCYSTS. Z. Ye, R. N. Clarke, R. Bodine, N. Zaninovic, L. Veeck Gosden. Weill Medical Coll of Cornell Univ, New York, NY.

OBJECTIVE: Pregnancy rates following frozen embryo transfer (FET) may be influenced by the stage of embryonic development at the time of cryopreservation. This limited analysis was undertaken to determine whether or not slow-growing preembryos that do not form blastocysts until Day 7 after harvest are capable of establishing clinical pregnancies.

DESIGN: Retrospective analysis of Day 7 frozen blastocyst transfer results from September 2005 through February 2006.

MATERIALS AND METHODS: Blastocysts were frozen on Day 7 after documenting slow growth characteristics on earlier days. Glycerol and sucrose were used as cryoprotective agents according to the methods of Veeck et al. (Fertil Steril 2004; 82:1418), and were thawed on the day of their replacement in either a natural or programmed replacement cycle. Thawed blastocysts were cultured approximately 5 hours before transfer.

RESULTS:

	Day 7 Blastocysts (%)
No. Cycles	6
Survived/Thawed	12/17 (70.6)
Clinical Preg/Transfer	3/5 (60.0)
Ongoing Preg/Transfer	3/5 (60.0)
Sacs/ No. Transferred	3/12 (25.0)

CONCLUSION: In an earlier study of blastocysts frozen on Days 5 and 6, survival, clinical pregnancy, ongoing pregnancy, and implantation rates were 76.3%, 59.2%, 50.2%, and 38.6%, respectively (Fertil Steril 2004; 82:1418). The results in this limited series of thaw cycles imply that Day 7 blastocysts are fully capable of surviving and establishing clinical and ongoing pregnancy rates comparable to blastocysts frozen on earlier days. Although the number of Day 7 thaws is too limited to draw significant conclusions, results are encouraging enough for this group to continue routinely culturing all embryos until at least Day 7 after harvest. In the last six months at our Center, 181 Day 7 blastocysts have been frozen in 70 cycles; an update will be presented next year.

Supported by: The Center for Reproductive Medicine and Infertility.

P-179

INTENTIONAL BLASTOCYST CRYOPRESERVATION PROGRAM FOR WOMEN WITH MULTIPLE FAILURES OF CONCEPTION AFTER IVF/ICSI-ET. C. Oka, T. Mukaida, T. Goto, K. Takahashi. Tokyo HART Clinic, Tokyo, Japan; Hiroshima HART Clinic, Hiroshima, Japan.

OBJECTIVE: To investigate clinical usefulness of total blastocyst(BL) vitrification and subsequent warmed embryo transfer(ET) into the endometrium prepared with hormone replacement therapy(HRT) for patients who had multiple failures of conception with IVF/ICSI-ET.

DESIGN: Prospective study.

MATERIALS AND METHODS: One hundred sixty women who had had multiple failures of conception with fresh ET and/or vitrified BL-ET were entered in this study. Mean age of women was 37.3 years and mean number of previous ET was 5.1. The study was carried out between January 2004 and December 2005. All women received standard ovarian hyperstimulation and IVF/ICSI. On day 5 after insemination, only good quality BLs were vitrified with cryoloop where one or two BL were stored in each according to quality of BL. In subsequent cycle, women received daily oral estrogen (E, Premarin 3.75mg or Estrace 4mg/day) from day 3 of menstrual period. Progesterone (P) 50mg IM and HCG 10,000 IU were given on the day when endometrial thickness reached 9 mm. BL-ET was performed 5 days after initiation of P injection. The best quality of BL was chosen for ET. HCG was measured 14 days after initiation of P injection. Pregnancy was determined with the presence of fetal heart beat (FHB). E and P administrations were continued until the end of 9 weeks of gestation after confirmation of rising E and P concentrations.

RESULTS: Mean number of oocyte retrieval and vitrified BL were 10.4 and 2.7, respectively. Patients were divided in 3 groups by age. Group I included 28 women of 34 years or younger, group II included 86 of 35-39, and group III included 46 of 40 or older. All women could have BL-ET. In group I, 48 BLs were transferred (mean number 1.7) and 22 FHB were confirmed (implantation rate 45.8%) in 18 women (pregnancy rate 64.3%). In group II, 136 BLs were transferred (mean number 1.6) and 38 FHB were confirmed (27.9%) in 31 women (36.0%). In group III, 65 BLs were transferred (1.4) and 9 FHB were confirmed (13.8 %) in 9 women (19.6%).

CONCLUSION: We have reported high pregnancy rate with vitrified BL using cryoloop. Many of those, however, were derived from patients who produced many surplus good quality BL. Therefore, they could become successfully pregnant with vitrified BL after failure with fresh ET. In this study group who had multiple failures of conception after BL-ET including vitrified BL-ET, women may have had no opportunity to have the best quality BL -ET in endometrium with HRT. In these women, we hypothesized that endometrium prepared with HRT is better for implantation than that with natural or stimulation cycle. Some of them had had vitrified BL-ET with HRT endometrium, but had not had the best one transferred because we tend to transfer the better quality embryo in fresh cycle and/or these women do not produce so many surplus BLs. We concluded that intentional blastocyst cryopreservation program is effective for those who had multiple failures of conceptions after BL-ET. Although similar grades of BL were vitrified and transferred, implantation and ongoing pregnancy rates were significantly lower in women older than 40 years.

Supported by: None.

P-180

IMPORTANCE OF 17-β ESTRADIOL AND ENDOMETRIAL THICKNESS IN ASSISTED REPRODUCTIVE CYCLES WITH EMBRYO TRANSFER AFTER OOCYTE THAWING. T. Bartolotti, M. F. Camerani, V. Felletti, E. Fogli, M. De Paola, G. Sintini. AUSL RA, Lugo RA, Italy.

OBJECTIVE: Importance of 17-β estradiol and endometrial thickness in assisted reproductive cycles with embryo transfer after oocyte thawing.

DESIGN: Interest in oocyte cryopreservation has recently increased, especially in Italy following the introduction of the Law 40 of February 19th 2004 that forbids human embryo experimentation. Today, biological research is concentrated in new protocols application to improve survival and fertilization rates of oocytes; nevertheless, the clinical efficiency of cryopreservation method is still controversial. By this way, to perform embryo transfer at correct endometrial timing could represent an essential viable of intervention.

MATERIALS AND METHODS: 152 oocytes were frozen by slow cooling protocol (0.1M Sucrose); 64 of these were thawed by fast thawing procedure (0.2M Sucrose) and 13 embryo transfer were performed in women having mean age 33.8 years. 609 oocytes were frozen by slow cooling protocol (0.3M Sucrose); 64 of these were thawed by fast thawing procedure (0.3M Sucrose) and 38 embryo transfer were performed in women having mean age 34.8 years . Endometrial growth was obtained by estrogenic oral or transdermic administration and intramuscular progestinic therapy and was observed by ultrasound transvaginal scanning and hormonal haematic assays (17-β estradiol). Only patients having endometrial