extenders/cryoprotectants need to be explored to lessen the effects of lipid peroxidation during gamete storage.

P-85

Blastocyst cryopreservation: An update. Barry Behr, Janice Gebhardt, Amin A. Milki. Stanford Univ Sch of Medicine, Stanford, CA.

Objective: Cryopreservation at the blastocyst stage has remained controversial in IVF. Many programs routinely cryopreserve extra embryos at the cleavage stage and shy away from blastocyst cryopreservation. We had previously reported our preliminary successful experience with routine cryopreservation at the blastocyst stage. The present study updates our findings with blastocyst transfer in a larger series of patients.

Design: Retrospective study.

Materials and Methods: We reviewed the clinical records of patients at our institution undergoing frozen embryo transfer (FET) of blastocysts over a three year period. Patients having a day three embryo transfer had remaining embryos cultured to day 5/6 for potential cryopreservation. Patients with more than three 8-cell embryos on day 3 were offered fresh blastocyst transfer on day 5 with additional embryos cryopreserved on day 5 and/or day 6. Only blastocysts with distinct inner cell mass and well developed trophoblast were cryopreserved utilizing the Menezo two-step protocol with mHTF and 20% SSS (Irvine Scientific, Santa Ana, CA) as the base medium. Blastocysts were thawed using the two-step thaw protocol with the same base medium. Patients were transferred in a natural cycle by the same physician with an hCG trigger when the lead follicle reached 17mm in diameter. Embryo transfer was performed 7 days after hCG and progesterone supplementation began 4 days after hCG.

Results: Out of 163 thaw transfers, we achieved a 25% (40/163) ongoing/delivered pregnancy rate. Seventy four patients had FET of day 5 blastocysts of which 27% (20/74) achieved a viable pregnancy and 89 patients had FET of day 6 blastocysts of which 22% (20/89) achieved a viable pregnancy. The difference in success rates between day 5 and day 6 FET's was not significant ($p>0.05$). The mean number of blastocysts transferred was 2.0. The mean age of the patients was 36 years.

Conclusion: This updated series confirms our initial experience with blastocyst cryopreservation. We believe that this modality offers the option of cyropreserving embryos that have proven their viability prior to freezing and thus avoids unnecessary expenditure of effort and finance on cleavage stage embryos that would have potentially arrested in culture. Extended culture could decrease the number of frozen embryos held in storage while maintaining pregnancy rates and allows embryos that don't meet day 3 criteria for cryopreservation to develop to blastocyst and have the opportunity to be cryopreseved. These data also support our initial findings that blastocysts have comparable pregancy potential whether frozen on day 5 or day 6 and may speak to the role of synchronizing the transfer with the endometrium in the thaw cycle.

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A healthy female born after ICSI of a cryopreserved oocyte and cryopreserved spermatozoa banked prior to radiotherapy in a patient with a seminoma: A case report. Judith Notrica, Laura Kanzepolsky, Andrea Divita, Fernando Neuspiller, Ester Polak de Fried. CER Medical Institute, Capital Federal, Argentina.

Objective: Banking of semen specimen prior to radio/chemotherapy allows patients to preserve future fertility. Oocyte cryopreservation may be an alternative, specially now that certain efficacy of the technique has been demonstrated.

There are very few publications about the use of thawed sperm from patients who have undergone chemo/radiotherapy and then gone on to perform ART with cryopreserved oocytes. To the best of our knowledge this is the first case of ICSI with cryopreserved oocytes and banked spermatozoa due to a seminoma. The objective of this report is to inform the birth of a healthy baby after the application of this technique

Design: Case report.

Materials and Methods: A 30-year-old male with diagnosis of seminoma was referred to our institute for semen banking before radiotherapy. Six vials of sperm were banked. After oncologic treatment was done, his partner, a 23-year-old woman, underwent controlled ovarian hyperstimulation with gonadotrophin therapy (rFSH 200 IU/day) with leuprolide acetate (0.5 mg/day) desensitization from the previous midluteal phase. 17 oocytes were retrieved, one was at the VG stage, two were inseminated by ICSI, obtaining two embryos; 14 MII oocytes were frozen according to the protocol previously published (Fertil Steril., 1997, 69, 555-557). The woman presented moderate ovarian hyperstimulation syndrome and the cryopreservation of the embryos was indicated for a future transfer.

Results: After endometrial preparation, good quality embryos were thawed and transferred; this process led to a single pregnancy; the woman suffered from eclampsia during the 28th week of gestation and cesarean surgery was performed. A preterm female of 1000 grams was born and died two weeks after the delivery. One year and a half later a second procedure was started; all the frozen oocytes and one vial of semen were thawed. 8 out of 14 oocytes survived and were microinjected; two became fertilized and one cleaved embryo of good quality was transferred.

Pregnancy was achieved and delivery was performed by cesarean surgery at 36 weeks of gestation, a healthy female was born with a birth weight of 2800 grams.

Conclusion: Oocyte cryopreservation is a useful tool when oocyte recruitment is high and there is no intention to store supernumerary embryos; in young women at risk of ovarian hyperstimulation this procedure offers a solution because gametes can be used or not, so no legal nor ethics conflicts may occur. This technique combined with banked sperm from an oncologic patient is a novel procedure for couples in need of differed parenthood

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Apoptosis in cryopreserved ovarian tissue—a tool of evaluation cryopreservation utility. Eli Rimon, Tania Cohen, Avraham Amsterdama, Ami Amit, Joseph Lessing, Fuad Azem. Tel Aviv Univ, Tel Aviv Medical Ctr, Tel-Aviv, Israel; Weizman Institute of Science, Rehovot, Israel.

Objective: Ovarian tissue cryopreservation (OTCP) is an emerging technology, offered for young cancer patients prior to chemotherapy. The optimal techniqe of OCTP is not determined yet. It has been demonstrated that 40-50% of preserved tissue is damaged following freezing and thawing.

The aim of this study was to examine the utility of OCTP by investigating apoptosis of follicles in frozen thawed ovarian tissue.

Design: Prospective study.

Materials and Methods: Ovarian tissue samples were obtained from 6 women with cancer who underwent cryopreservation of ovarian tissue prior to chemotherapy. Ovarian cortex samples measuring 2mm X 5 mm were removed by laparoscopy. The study was approved by the institutional ethical committee and the women signed an inform consent. One tissue sample was evaluated for apoptosis immediately following removal and served as a control. A second tissue sample was frozen, thawed and then evaluated for apoptosis. Light microscopy using hematoxylin and eosin (H&E)-stained paraffin slides and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labelling (TUNEL) methods were used to confirm the incidence of apoptosis. The number of apoptotic follicles is scored on micrograph of at least 4 random sections stained by TUNEL. Follicles were defined apoptotic if they contain at least 6 cell nuclei per section, which are positive to the TUNEL reaction.

Results: Apoptosis was demonstrated in 70 ± 6 % of follicles in frozen-thawed ovarian samples compared to 25 ± 12% in the control samples. The same results were obtained with H&E.

Conclusion: Higher incidence of apoptosis has been demonstrated in ovarian follicles in frozen-thawed ovarian tissue compared to unfrozen ovarian tissue. This findings reflect a severe tissue damage, which occurs during the freezing thawing process. The investigation of tissue apoptosis may be used as a tool in evaluating the utility of various freezing-thawing prtocols.

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Comparison of slow freezing in the cleavage stage and ultra-rapid vitrification of blastocysts. Miho Tanaka, Atsumi Yoshida, Hiroki Suzuki, Akiko Takahashi, Keiko Seida, Tomomi Fujimori. Kiba Park Clin, Tokyo, Japan.

Objective: Freezing technology for blastocysts is an absolute necessity at facilities that perform ART. Ultra-rapid vitrification of blastocysts, which