therefore conducted nuclear transfer between in vitro matured metaphase-II oocytes.

DESIGN: The fertilzation rate, cleavage rare and blastocyst formation rate were investigated following nuclear transfer.

MATERIALS AND METHODS: Recipient oocytes were derived from immature oocytes in ICSI treatment and cultured in vitro until the first polar body extrusion was observed. Donor oocytes were prepared from IVF or ICSI patients who had consented to participate in these experiments. Two oocytes were placed in a microdrop containing 5 microgram/ml of cytochalasinB (CCB). A 10-12 micrometer innerdiameter glass pipette was inserted into the perivitelline space and the cytoplasm contatining M-II chromosome was aspirated. The karyoplast of donor oocytes was removed using the same procedure that was used for recipient oocytes and transferred into the perivitelline space of an enucleated donor oocyte. Insertion of the karyoplast, the grafted oocyte was washed for 60 minutes in HTF medium to remove CCB, and then transferred in Zimmerman cell fusion medium. Membrane fusion between oocyte and karyoplast was facilitated by electrical stimulation (10V/sec AC + 15V/45 μsec DC) with an electro cell fusion generater (LF 101). After fusion, the constructed oocytes were cultured in HTF medium for 2 hours and ICSI was performed.

RESULTS: The percentage of identification of M-II chromosome was in 42 of the 44 freshly ovulated oocytes (95.5%) and in 38 of the 40 in vitro-matured oocytes [92.7% (55/55)]. The M-II karyoplast was removed successfully in 38 of 42 (90.5%) of the donor oocytes and 45 of 51 (88.2%) of the recipient oocytes. All of 38 karyoplasts of recipient oocytes were replaced in periviteleine space of enucleated donor oocytes and 30 of these 78.9% were fused to form a reconstituted oocyte. The fertilization rate, cleavage rate and blastocyst formation rate following ICSI for constructed oocytes and recipients oocytes were (76.7% (23/30), 66.7% (20/30), 33.3% (10/30))respectively. Chromosomal analysis of 4 embryos following nuclear transfer were diploid sets of 46 chromosome.

CONCLUSIONS: These results suggest that normal embryonic development can occur after the transfer of karyoplast between in vitro-matured metaphase-II oocytes.

Supported by: None.

Wednesday, November 12, 2008
3:00 pm

O-285

LASER ACUPUNCTURE BEFORE AND AFTER EMBRYO TRANSFER IMPROVES ART DELIVERY RATES: RESULTS OF A PROSPECTIVE RANDOMIZED DOUBLE-BLINDED PLACEBO CONTROLLED FIVE-ARMED TRIAL INVOLVING 1000 PATIENTS. J. L. Fratterelli, M. R. Leondires, K. Fong, A. Theall, S. Locatelli, R. T. Scott. Reproductive Medicine Associates of New Jersey, Morristown, NJ.

OBJECTIVE: Acupuncture has been advocated for the treatment of infertility, including immediately prior to and after transfer. Unfortunately, data are conflicting and adequate studies with appropriate controls are lacking. This study evaluates 2 forms of acupuncture on outcomes in ART patients.

DESIGN: Prospect randomized double blind and placebo controlled.

MATERIALS AND METHODS: On the day of transfer, participants were randomly assigned to a study group; needle acupuncture (AC), laser acupuncture (LZ AC), sham laser acupuncture (LZ sham), relaxation (RX), or no treatment (NT). The AC and LZ AC puncture groups were considered treatment groups, the RX controls for the additional rest before and after transfer, and NT is the non-intervention group. Most significantly, the LZ Sham group provided an important control group. The laser acupuncture device was randomly preprogrammed per case to either fire (and provide LZ AC) or to not fire and thus provide a true double blind control group (LZ sham). It was not possible for the patient or acupuncturist to know if the laser fired. No contact oocurs with the patient in laser acupuncture so there is no acupressure effect or contact with the wrong meridians. All treatments were administered for 25 minutes before and after embryo transfer. Outcomes were compared by Chi-square and multiple logistic regression analysis to control for the potential confounders including female age, embryo quality, and day of transfer (Table 1).

RESULTS: All treatments were well tolerated. No differences in terms of patient demographics, cycle type, stimulation outcomes, embryo number and quality, day of embryo transfer, transferring physician, or acupuncturist were found between the 5 study groups. Implantation rates were significantly improved with laser acupuncture. Traditional needle acupuncture had outcomes equivalent to the 3 control groups. Subanalyses of patient age and embryo transfer day produced similar findings with laser acupuncture enhancing outcome rates.

CONCLUSIONS: This large prospective randomized and well controlled study consistently demonstrated benefit to LZ AC. Treatment was well tolerated and significantly improved implantation rates.

TABLE 1. Clinical Outcomes (%)

Rates	AC (n=200)	LZ AC (n=202)	LZ Sham (n=198)	RX (n=203)	NT (n=197)	*P* Values
Implantation	28.9	33.7	26.8	24.9	30.2	< 0.05
Chemical Pregnancy	61.5	60.9	53.0	53.7	60.4	0.22
Clinical Pregnancy	51.5	54.5	43.9	45.3	50.3	0.19
Ongoing Pregnancy/ Delivery	39.0	42.1	35.4	37.4	39.6	0.71

Supported by: EMD Serono.

Wednesday, November 12, 2008
3:15 pm

O-286

EFFECT OF MODIFIED QUARTER LASER-ASSISTED ZONA THINNING (MQLAZT) ON PREGNANCY AND IMPLANTATIONS RATES IN CRYOPRESERVED EMBRYOS: A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL. S. Debrock, C. Spiessens, D. Willemen, K. Peeraer, T. M. D'Hooghe. Leuven University Fertility Center, Leuven, Belgium.

OBJECTIVE: Freezing/thawing cycles can impair the successful hatching process. The hypothesis was tested that the clinical implantation rate (CIR) per embryo transferred and the clinical pregnancy rate (CPR) per embryo transfer (ET) is higher after ET of frozen-thawed embryos with thinned zona pellucida (ZP) after mQLAZT when compared to ET of frozen-thawed embryos without mQZLAT.

DESIGN: Prospective randomized controlled study (RCT) in an academic tertiary setting.

MATERIALS AND METHODS: Patients with frozen embryos, recruited for this study after informed consent, were randomized at the time of thawing to a study group with mQLAZT or a control group without mQLAZT. After thawing, embryos were kept in culture for 24 hours to evaluate further cleavage. mQLAZT was performed 5 min to 1 hour before ET using a laser system (MTG, Germany) on an inverted microscope. The ZP was thinned to about 1/3 of the depth and for a distance of 120° from the 1 o'clock position to the 5 o'clock position. Only results of cycles with equal number of embryos for ET and mQLAZT were included. Primary outcome variable was CIR per embryo transferred (with fetal sac on ultrasound at 6-8 weeks of pregnancy). Power calculation (beta = 0.8, alpha < 0.05) revealed that 270 embryos were needed in each group. Other outcome variables were ongoing (at 12 weeks pregnancy) IR per embryo transferred, CPR and ongoing PR per ET. Data were analysed by Mann-Withney test and Chi square analysis and considered significant if $P < 0.05$. NS is not significant.

RESULTS: A total of 526 thawing cycles were randomized to either the study (N=263) or control group (N=263). Reproductive outcome data were available for 246 cycles in the study and 260 cycles in the control group for the following reasons: technical laser problems (study: N=2), no mQLAZT performed due to risk to harm embryo with thin ZP (study: N=6), N embryos for ET was not equal to N embryos with mQLAZT (study: N=5), mQLAZT was performed or not performed by mistake (study: N=3,