Design: A randomized controlled clinical trial.

Materials and Methods: Twenty-eight patients with PCOS who previously failed to conceive despite 3 ovulatory cycles of CC were randomized to 3 groups; (i) OI with CC (100 mg/d) and IUI (n:8), (ii) OI with low dose gonadotopins and IUI (n. 10), (iii) OI with low dose gonadotropins (n:10). Fifteen patients with PCOS who previously failed to develop preovulatory follicles in 3 cycles on CC (100–200 mg/d) were randomized to 2 groups; (i) OI with low dose gonadotropins (n:7), (ii) OI with low dose gonadotropins and IUI (n:8). In IUI cycles, two IUI procedures were done and sperm was prepared using a two-step wash procedure.

Results: In patients with failure to conceive despite ovulation on CC, ongoing PRs per started cycles were 4/8 (50%) In CC+IUI group, 6/10 (60%) in low dose gonadotropin+IUI group, and 2/10 (20%) in low dose gonadotropin only group ($p<0.05$). In patients with failure to develop follicles on CC, ongoing PRs per started cycles were 2/19 (10%) in low dose gonadotropin only group, and 4/19 (21%) in low dose gonadotropin+IUI group ($p>0.05$).

Conclusions: In case of anovulatory resistance to CC, we offer combination of low dose gonadotropin regimen with IUI in order to decrease the number of cycles with costly gonadotropins to achieve a pregnancy. In case of non-conceptional resistance either to CC or gonadotropins, IUI should be tried before stepping up to a higher line of treatment.

## REPRODUCTIVE LABORATORY TECHNOLOGY

Monday, October 23, 2000

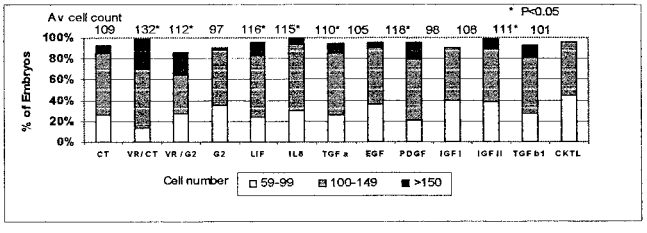
**P-156**

**Effect of Growth Supplementation on Blastocyst Development in Sequential Human Blastocyst Culture Medium.** N. Desai[1], J. Lawson, J. Goldfarb. Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH.

Objective: While new sequential media formulations for human blastocyst culture have given excellent pregnancy rates in initial trials with pre-selected patient populations, there is some question as to whether all patient embryos would be adequately supported. Augmentation of development through growth factor supplementation may be one means to improve current formulations. In this study, blastocyst development in coculture and in sequential culture medium with different growth factor additives was compared.

Design: Computer assisted image analysis of morphology along with immunoflourescent staining of cell nuclei.

Materials and Methods: Zygotes were isolated from B6C3F1 mice and cultured overnight. Two cell embryos were pooled and randomly distributed to Gardner's G1.2/2.2 medium supplemented with individual growth factors (GF) or a GF cocktail. The following growth factors were tested: 1) LIF (1000 pg/ml), 2) IL-6 (1000 pg/ml), 3) IGF I (30 ng/ml), 4) IGF II (1 ng/ml), 5) TGF $\alpha$ (2 ng/ml), 6) TGF $\beta_1$ (2 ng/ml), 7) EGF (4 ng/ml) 8) PDGF (1000 pg/ml). The GF cocktail consisted of TGF$\alpha$, EGF, IGF II and PDGF. All treatments were contrasted to coculture on Vero cells. Embryo development and morphology in each culture regimen was monitored for 72 hrs. Cell nuclei were then labeled with bisbenzamide for cell counting.




Results: Blastocyst formation and hatching rates were similar between test groups. The average cell count was higher with coculture or the addition of specific growth factors. Coculture resulted in a greater percentage of blastocysts with more than 150 cells.

Conclusions: Blastomere number in embryos cultivated in G1.2/2.2 can be modulated by addition of specific growth factors and by coculture with Vero cells.

**P-157**

**Pregnancy Following the Transfer of Ooplasm Derived from Donor 3PN Eggs into Recipient Oocyte.** J. H. Moon, S. H. Yoon, H. G. Yoon, S. P. Park, J. H. Jung, J. H. Lim. Maria Infertility Clinic, Seoul, Korea.

Objective: The aim of this study is to examine if ooplasm derived from three pronucleus (3PN) eggs could be used to improve the fertilization and the subsequent embryonic development in patients with history of poor fertilization and/or defective embryonic development.

Design: After transfer of ooplasm derived from donor 3PN eggs into the recipient oocyte, their fertilization, developmental quality and subsequent pregnancy were evaluated.

Materials and Methods: The study was performed in case of the repeatedly IVF-failed (3 or more) patients (n=23, mean age = 35 years) with history of poor fertilization and/or defective embryonic development.

The procedure of ooplasmic transfer was as follows. A small volume of ooplasm from a donor egg was first sucked into an injection pipette, and then immobilized sperm was sucked into the same pipette to be placed on top of the ooplasm. The harvested ooplasm and a sperm were injected into a recipient MII stage oocyte at the direction of 3 p.m. A polar body could generally be observed in the direction of 6 or 12 p.m., which indicates successful fertilization. The fertilized eggs were co-cultured with cumulus cells in YS medium containing 10% hFF for 3 days. Embryo morphology was scored into 4 grades (grade 1 to grade 4) according to the regularity of blastomeres and the amount of anuclear fragments. Embryos were then transferred into the uterus.

Results: Three pronucleus (3PN) eggs were prepared from 38 donors. Ooplasmic transfers were performed in the oocytes of 23 patients, resulting in 77.4% (96/124) of fertilization. The rate of good quality embryos containing grade 1 and 2 was 38.8%. Following their transfer, the pregnancy was established in three patients (13%, 3/23) with history of poor fertilization and/or defective embryonic development.

Conclusions: The ooplasm derived from three pronucleus (3PN) eggs may provide as a useful source of ooplasm for transfer into recipient oocytes, probably eliminating the ethical problems. Further studies are necessary to examine the possibility of genetic abnormalities caused by 3PN-ooplasmic transfer.

**P-158**

**Blastocyst Development Is Significantly Decreased but Maintains a High Implantation Rate in Cases of Severe Male Factor Infertility.** M. H. Majercik, W. K. Firisin, M. Abaé. Center for Advanced Reproductive Endocrinology, Plantation, FL.

Objectives: Blastocyst development and other embryology laboratory data were compared in patients undergoing IVF with ICSI for severe male factor infertility, mild to moderate male factor infertility treated with ICSI, and non-male factor infertility patients undergoing conventional IVF.

Design: Retrospective comparative study of 50 consecutive IVF cases with embryo transfer at the blastocyst stage.

Patients and Methods: All patients who have undergone blastocyst transfer as part of their IVF cycle at our center, ranging in age from 26–40, were divided into 3 groups: **Group I:** Patients with normal sperm parameters whose cohort of eggs underwent IVF with conventional insemination (N=22; mean sperm count 120 $\pm$ 61.1 million/mL, motility 60.5 $\pm$ 11.8%, and strict normal morphology 9.6 $\pm$ 2.3%); **Group II:** Patients whose eggs were treated with ICSI for mild to moderate male factor infertility (N=18; mean sperm count 73.7 $\pm$ 59.2 million/mL, motility 45.7 $\pm$ 11.8%, and strict normal morphology 4.5 $\pm$ 2.0%); **Group III:** Patients requiring ICSI for treatment of severe male factor infertility (N=10; mean sperm count 8.5 $\pm$ 3.0 million/mL, motility 15.9 $\pm$ 11.9%, and strict normal morphology 1.4 $\pm$ 0.9%). Group III also contained patients who required testicular biopsy for obstructive azoospermia and spinal cord-injured patients requiring electroejaculation, whose sperm parameters are not included in the means reported above. Controlled ovarian hyperstimulation was performed using standard protocols, and embryos were maintained in sequential culture media supplied by IVF Science (IVF-50, S1/G1, S2/G2). Statistical analysis was performed using ANOVA.