had spontaneous pregnancy with normal term delivery. Two women conceived but had early repeated miscarriages. Five women, after about 3 years of infertility, underwent assisted reproduction treatments.

Conclusions: PCOS is generally assumed to find its pathogenetic moment during puberty and adolescence. During adolescence young healthy subjects with irregular cycles, not infrequently show a clinical spectrum of symptoms very similar to that found in adult PCOS, particularly regarding androgen levels, excessive LH secretion with a desynchronized LH rhythm, and the polycystic ovarian structure. In some girls this PCOS-like condition may represent an alternative maturative pathway toward normal adult function but our investigation showed that in most subjects it crystallizes in a claimed adult PCOS condition. Therefore, in the development process of the reproductive function, postmenarchal anovulation is probably the crucial step for the onset of pathology. It is noteworthy that at least half of the subjects of our study presently suffer from infertility. Persistent irregular anovulatory cycles in adolescence may well represent an early negative prognostic sign for fertility.

Wednesday, October 25, 2000
3:00 P.M.

O-241

Criteria for the Prevention of High Order Multiple Births Following Ovulation Induction with Gonadotropins. [1,2,3]N. Gleicher, [4]D. Oleske, [5]I. Tur-Kaspa, [2]A. Vidali, [1,3]V. Karande. The [1]Center for Human Reproduction (CHR)—Illinois, [2]CHR—New York, [3]the Foundation for Reproductive Medicine, Illinois, [4]Department of Preventive Medicine and Health System Management, Rush—Presbyterian/St. Lukes Medical Center, Chicago, Illinois, [5]the IVF Unit, Barzilai Medical Center, Ben Gurion University, Ashkelon, Israel.

Objectives: We previously reported (ASRM, 1999) that the occurrence of high order multiple births, defined as triplets or higher order multiples, was unpreventable in 1.2% of ovarian stimulation cycles using gonadotropins and utilizing widely accepted criteria of cycle monitoring with ultrasound and estradiol. This follow-up study was conducted to determine whether new cycle monitoring criteria could be established to reduce the incidence of high order multiples.

Design: Retroactive analysis of 4035 previously reported cycles and calculation of receiver operator characteristic curves 'and ordinal logistic regressions for peak estradiol levels (pE_2), total number of follicles (tF) and follicles > = 16 mm size (F-16).

Materials and Methods: 4035 consecutive gonadotropins stimulation cycles, mostly accompanied by intrauterine inseminations, were assessed for pE_2, tF, and F-16 at time of hCG administration. The data was retrieved from a computerized data file. Statistical analysis was performed by one of the authors (D.O.) using standard statistical packages. The occurrence of no pregnancy, low order pregnancy (singleton, twin) and high order pregnancy (triplet or higher order) were also obtained from the computerized data file. Significance was defined as $p < = 0.05$.

Results: Neither number of F-16 nor the discriminatory usage of standard serum E_2 cut off concentrations of 2,000 pg/ml (7,342 pmol/L) or 2,500 pg/ml (9,178 pmol/L) were associated with high order pregnancies. However, increasing tF and increasing peak serum E_2 concentrations, each correlated significantly with the risk of high order pregnancies ($p<0.0001$), as did younger female age ($p=0.012$). Peak serum E_2 concentration quintiles and total number of follicles, which represented optimal strata for predicting higher order births, suggested that a peak serum E_2 concentration of >/= 1,385 pg/ml [5,084 pmol/L; multivariate odds ratio 1.88; (95% confidence interval, 1.27–2.80 and >/= 7 follicles; multivariate odds ratio 2.14; 95% confidence interval, 1.16–3.930] represented thresholds of risk for high order births.

Conclusions: (1) Especially in young women, a decrease in currently used peak serum estradiol level thresholds may reduce the incidence of high order births. (2) Such a reduction would, however, also result in a significant reduction of overall pregnancy rates. (3) Currently used ultrasound criteria are basically worthless in predicting risk for high order multiples. (4) It is very questionable whether any stimulation criteria for gonadotropins cycles can be devised which offer reasonable pregnancy rates and a low high order multiple pregnancy risk. (5) These observations further suggest that IVF should be considered earlier in a traditional infertility treatment algorithm if the occurrence of high orders multiples is of concern.

Wednesday, October 25, 2000
3:45 P.M.

O-242

Effects of Tamoxifen (Tx) on Endometrial Thickness and Pregnancy Rates in Women Undergoing Superovulation with Clomiphene Citrate (CC) and Intrauterine Insemination (IUI). A. Saleh, M. M. Biljan, S. L. Tan, T. Tulandi. McGill Reproductive Center, Royal Victoria Hospital McGill University, Montreal, Canada.

Objective: CC is commonly used to either provoke ovulation in anovulatory patients, or to increase the number of pre-ovulatory follicles in ovulatory patients prior to IUI. However, pregnancy rates following induction with CC and IUI are lower than expected for the number of follicles produced. This may be a result of the anti-estrogenic effect of CC on the endometrium. Tx has been previously been shown to act as an estrogen agonist on endometrial cells causing, after prolonged use, endometrial hyperplasia. In this study we investigated the potential positive effect of Tx on the endometrium, and pregnancy rates in patients treated with CC prior to IUI.

Design: Retrospective study.

Materials and Methods: Patients were treated with either CC alone or a combination of CC with Tx. CC was given for five days in the early follicular phase in doses between 50 and 150 mg daily, depending on the patient's weight and response in a previous cycle. In patients co-treated with Tx, Tx doses were administered simultaneously with CC. In the first cycle patients were given 20 mg of Tx daily. Thereafter, the dose was increased in a stepwise fashion up to 80 mg daily, depending on the endometrial thickness achieved in the previous cycle. Ultrasound scans were performed between day 1 and 5, on day 9 of menstrual cycle, and as required thereafter, until the dominant follicle reached 18 mm in mean diameter. Ovulation was then triggered with 10,000 in hCG and IUI were performed 24 and 48 hours later.

Results: All 594 IUI treatment cycles conducted from October 1998 to October 1999 on patients who were <40 years of age and had no tubal damage were included in the analysis. Of these, in 448 cycles (243 patients) CC only was used, while in 146 cycles (87 patients) Tx was added to the induction regimen. There was no difference in age, cause or length of infertility, or semen parameters, at the time of insemination in the two groups. Additionally, at the time of hCG administration, no difference was observed in the total number of follicles ($p=0.13$) or number of follicles >14 mm ($p=0.39$). In the cycles where Tx was added, on the day of hCG administration the endometrium was significantly thicker than in the cycles where CC was used alone ($p=0.0019$). Additionally, a significantly higher pregnancy rate per cycle (16.4 vs. 9.8% Odds Ratio = 1.8 95% CI=1.1–3.2) was achieved in patients co-treated with Tx.

Conclusions: The addition of Tx to an ovulation induction with CC does not increase the total number of available follicles or the number of pre-ovulatory follicles. It does, however, probably by competing for same E_2 receptors, increase the endometrial thickness and enhances endometrial implantation potential. Co-treatment with Tx should, therefore, be considered in all patients undergoing ovulation induction with CC, or at least in patients who have previously demonstrated a poor endometrial development following treatment with CC.

Wednesday, October 25, 2000
4:00 P.M.

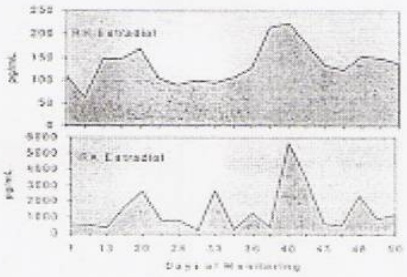
O-243

Restoration of Ovarian Function After Autologous Transplantation of Human Ovarian Tissue in the Forearm. K. Oktay, B. A. Aydin, K. Economos, J. Rucinski. Department of Obstetrics and Gynecology, New York Methodist Hospital and Cornell University Weill Medical College, NY.

Objectives: Transplantation of both fresh and frozen-banked parathyroid glands in the forearm has been successfully performed for decades (NEJM 1976;29:57). In this trial, we sought to determine whether a similar technique could be adapted to auto-transplant ovarian tissue to salvage ovarian functions in case of radical surgery or cancer therapy.

Design: A prospective study of experimental surgery.

Materials and Methods: In the pilot case, normal ovarian tissue was harvested from a 33-year old woman at the time of a cystectomy, and 5×5 mm pieces of ovarian tissue were grafted in the brachioradialis muscle to test the feasibility of this site as a recipient. In the index case, both ovaries were removed from a 35-year-old woman with stage IIIB squamous cell cervical carcinoma, prior to pelvic radiotherapy. The ovarian cortex was prepared in 0.5 × 5 cm. strips and 16 strips were transplanted subcutaneously in the forearm.



Results: In the pilot case, 1–2 mm antral follicles were detected in the graft by high-frequency ultrasound four months after the transplant. In the index case, six weeks after the oophorectomy, the FSH and LH were 47 and 35 mIU/mL, respectively. Ten weeks after the transplant, the patient noticed a painless bulge at the site of the transplant. Ultrasound examination showed a dominant follicle of 12 mm in size and 4 other antral follicles with sizes ranging 5–7 mm. Estrogen replacement was discontinued. Serial ultrasound exams showed continual development of new antral follicles as large as 18 mm. 140 days after the transplantation and over 60 days of monitoring, mean ± SE hormone levels from the right hand (RH) vs. the right cubital fossa (RCF) were as shown in table I. RH E_2 measurements, which represent the peripheral hormone levels, showed 2 cycles over 50 days (top fig). E_2 levels from the RCF, which represent the immediate output of the graft, showed a higher frequency of surges (bottom fig). Serum P_4 fluctuated but remained <4 ng/mL. Within 80–120 days after the transplant, FSH ranged 11–18.1 (13.6 ± 0.54) but between 120–140 days after the transplant it fell to <10 (range: 6.5–9.1, 7.3 ± 0.8) (p=0.00001). Within the same comparison periods, LH fell from 18.3 ± 1.5 to 14.0 ± 2.1 but this did not reach statistical significance (p=0.1).

Table I.

	E_2 (pg/mL)	P_4 (ng/mL)	Test (ng/dL)	FSH (IU/L)	LH (IU/L)
RH	130 ± 9.2	0.45 ± 0.04	36 ± 1.5	11.4 ± 0.79	16.8 ± 1.28
RCF	1233 ± 289	1.0 ± 0.25	51 ± 4.2	11.6 ± 0.77	17.5 ± 1.3
P value	0.001	0.001	0.008	0.3	0.02

Conclusions: Heterotopic transplantation of ovarian tissue can result in antral follicle development and relatively normal peripheral E_2 levels. Ovulation could not be confirmed yet, but percutaneous egg retrieval will soon be performed for IVF. Interestingly, the cubital vein assumes the role of ovarian vein, opening the possibility of more sensitive monitoring of ovarian functions. Higher LH levels in the RCF compared to RH is intriguing and deserves further investigation.

(Supported in part by the ASRM-Serono Research Grant.)

Wednesday, October 25, 2000
4:15 P.M.

O-244

Regulation of Cell Adhesion Components During the Differentiation of Human Trophoblasts. M. Esposito, *K. Amin, G. Coukos, C. Coutifaris. Departments of Obstetrics and Gynecology and *Surgery, University of Pennsylvania Medical Center, Philadelphia, PA.

Objectives: It is hypothesized that specialized proteins involved in cell-cell adhesion, matrix degradation and cell signaling are critical to the differentiation of human trophoblasts. In this investigation, the cAMP regulation of adhesion related genes was explored during the differentiation of human trophoblasts.

Design: Basic science laboratory investigation approved by the Institutional Review Board.

Materials and Methods: BeWo choriocarcinoma cells were treated with 1.5 mM 8-Br-cAMP to promote syncytialization. At 72 hours, cells were harvested, total RNA was extracted and further processed to obtain poly A mRNA and ^{32}P labeled cDNA. The labeled probe was hybridized to the Atlas™ Human Cell Interaction Array and analyzed using software supplied by the manufacturer. Immunocytochemistry and northern analysis was utilized to confirm the expression of some of the regulated proteins in normal trophoblasts.

Results: 8 Br-cAMP treatment up-regulated the genes for matrix metalloproteinase 2 (870%), plasminogen activator inhibitor-1 precursor (840%), fibronectin receptor alpha 5 subunit (300%), and VE cadherin (260%). Northern hybridization further confirmed the up-regulation of the alpha 5 integrin subunit after exposure to the cAMP analog of both BeWo and non-transformed trophoblast cells. Conversely, there was down-regulation of focal adhesion kinase (53%), E-cadherin (56%), beta catenin (61%), integrin alpha 6 precursor (77%), desmoplakin 1 and 2 (84%), and desmoglein 2 precursor (85%). Immunocytochemical localization of desmoplakins and E-cadherin further confirmed these observations at the protein level.

Conclusion: This preliminary study demonstrates the regulation of several genes involved in cell-cell and cell-matrix interaction during the terminal differentiation of trophoblasts from mononucleated cells to syncytia. We conclude that cAMP-mediated signaling triggers the modification of cell adhesion complexes, activation of MMP-2 and FAK and leads to the syncytialization of human trophoblasts and the acquisition of a non-invasive, endothelial-like phenotype by the cells.

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Wednesday, October 25, 2000
4:30 P.M.

O-245

Differential Gene Expression in High Versus Low Endometrial Receptivity Status. [1,2]J. Martin, [3]S. Avila, [3]O. Gimenez, [1,2]A. Pellicer, [3]J. L. Castrillo, [1,2]C. Simon. [1]Department of Pediatrics, Obstetrics and Gynecology, School of Medicine, Valencia University, [2]Instituto Valenciano de Infertilidad Foundation (FIVIER), Valencia and [3]Centro de Biologia Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain.

Objective: Endometrial receptivity is a key element for embryonic implantation and appears to be closely associated with morphological and biochemical changes of endometrial epithelial cells (EEC). To gain knowledge on this area we have used endometrial cell lines that exhibit either pronounced adhesiveness (human endometrial cell line RL95-2 (R cells)) or markedly less receptive characteristics (HEC-1-A cell line (H cells)) compared to primary EEC. Our objective was to investigate the adhesive features of both cell lines and study their gene differential expression in order to link gene patterns to receptive versus non-receptive status.

Design: The adhesive features of R, H and normal EEC were investigated using an embryo adhesion assay. To establish a possible differential gene expression pattern linked to the endometrial receptivity, cDNA probes from the total RNA of both cell lines were generated and used as hybridization probes to compare the expression pattern by using DNA array technique.

Materials and Methods: To examine the differential receptive properties of R and H cells, adhesiveness of mouse blastocysts (n=286) to confluent monolayers of these cells was measured using an established adhesion assay. Gene expression patterns were studied in confluent monolayers of R and H cells after isolation of total RNA. To analyze differential gene-expression, ^{33}P cDNAs probes were generated from RNA samples of both cell lines using a mixture of gene-specific primers. The labeled probes were separately hybridized to two membranes (Atlas™ cDNA Expression Arrays) with 91 duplicate human cDNAs. After a high-stringency wash, the membranes were exposed to x-ray films and the differential hybridization patterns were compared.

Results: Embryo adhesion assays confirmed the receptive status of the cells analyzed ranging from high receptive (R cells with 81% of mouse