

observed only during the late secretory phase. Within the endometriosis group, the mean numbers of vessels with endoglin expression in stages  $\text{E}^0$  and  $\text{E}^1$  were not different from stages  $\text{E}^2$  and  $\text{E}^3$ .

**Conclusions:** This study shows the expression of endoglin in the eutopic endometrium of women with endometriosis is significantly increased and the increase is observed only in the late secretory phase. It is suggested from these findings that activation of angiogenesis in the eutopic endometrium might be a key factor in the pathogenesis of endometriosis.

#### P-114

**The effect of prolonged GnRH agonist (GnRHa) therapy on in vitro fertilization-embryo transfer (IVF-ET) cycle outcome in endometriosis (ENDO) patients: a multicenter randomized trial.** E. S. Surrey, K. M. Silverberg, M. W. Surrey, W. B. Schoolcraft. Colorado Ctr for Reproductive Medicine, Englewood, CO; Texas Fertility Ctr, Austin, TX; Reproductive Medicine and Surg Assoc, Beverly Hills, CA.

**Objective:** Evaluate the effect of a 3 month GnRHa course immediately prior to IVF-ET in ENDO patients with the hypothesis that prolonged suppressive therapy would improve cycle outcome.

**Design:** Prospective randomized trial in 3 tertiary care assisted reproductive technology programs.

**Materials/Methods:** Candidates for autologous IVF-ET with a primary diagnosis of ENDO surgically confirmed within 48 months of cycle initiation and without evidence of persistent endometrioma  $>2$  cm were randomized into 2 groups. Group I (23 patients) received monthly GnRHa (Lupron Depot, TAP Pharm., Waukegan, IL) 3.75 mg IM  $\times$  3 followed within 17 days by standard controlled ovarian hyperstimulation (COH) including SC GnRHa. Group II (23 patients) received standard COH employing either mid-luteal phase GnRHa downregulation or microdose flare regimens. Comparative COH response and IVF-ET cycle outcome were evaluated. Pregnancy (PR) and implantation (IR) rates were defined as sonographically visualized cardiac activity/ET procedure. Data analysis: Student's group t-tests and Chi square analysis where appropriate.

**Results:** Preliminary results presented below are expressed as mean  $\pm$  SEM unless otherwise indicated. ASRM ENDO scores were significantly higher in Gr. I ( $31.5 \pm 3.2$ ) than in Gr. II ( $20.1 \pm 2.9$ ) ( $P < 0.05$ ). There were no significant differences between the groups with regards to age (I:  $33.4 \pm 0.7$  vs. II:  $33.3 \pm 0.5$  yrs.), COH dose (I:  $42.2 \pm 3.3$  vs. II:  $40.8 \pm 2.3$  ampules), COH duration (I:  $10.0 \pm 0.2$  vs. II:  $10.0 \pm 0.4$  days), fertilization (I:  $63.2 \pm 4.0\%$  vs. II:  $63.6 \pm 4.7\%$ ), embryos transferred (I:  $3.3 \pm 0.2$  vs. II:  $2.9 \pm 0.2$ ). Trends towards higher PR (I:  $78.3\%$  vs. II:  $56.5\%$ ) and IR (I:  $41.6\%$  vs. II:  $2.8\%$ ) in Gr. I did not reach statistical significance.

**Conclusions:** The prolonged use of a GnRHa in ENDO patients prior to IVF-ET had no deleterious effect on COH despite having been administered to patients with more severe disease and may improve cycle outcome. Clear delineation of an appropriate subset of ENDO patients who would derive a clear benefit from this approach requires a larger sample size.

*Supported by: Grant from TAP Pharmaceuticals, Waukegan, IL.*

#### P-115

**Bcl-2 expression and apoptosis in endometrium of women with and without endometriosis.** J. Ding, J. Shen, N. Rana, D. P. Braun, W. P. Dmowski. Institute for the Study and Treatment of Endometriosis, Oak Brook, IL; Rush Medical Coll, Chicago, IL.

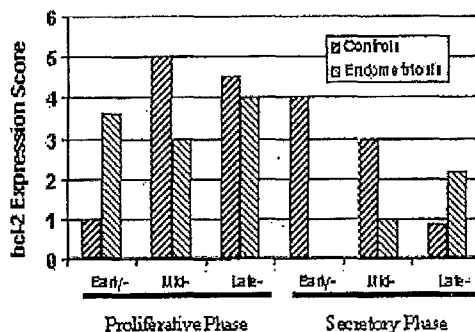
**Objective:** We have reported previously that spontaneous apoptosis in the uterine endometrium is reduced significantly in women with endometriosis compared to controls. This decrease was observed mainly during the early-proliferative and late-secretory phases. The present study investigated the correlation between apoptosis and Bcl-2 expression in uterine endometrium from women with and without endometriosis.

**Design:** Bcl-2 expression and apoptosis were measured in endometrial samples obtained from women with and without endometriosis at different phases of the menstrual cycle.

**Materials/Methods:** 52 paraffin blocks of uterine endometrium from patients who underwent laparoscopy for suspected endometriosis were retrieved from the pathology laboratory. Of these, 32 were positive and 20 were negative for endometriosis. Tissue sections were made for detection of

apoptosis and Bcl-2 expression by standard techniques. Apoptotic cells were identified by the TdT-mediated dUTP-biotin nick end-labeling technique (TUNEL assay) and the level of apoptosis expressed as an apoptotic index equivalent to the number of apoptotic cells per  $10 \text{ mm}^2$  area. Bcl-2 was detected using standard streptavidin-biotin peroxidase immunohistochemistry and the level of expression in both basal and functional layers was scored under a microscope. Correlation analysis between apoptotic index and Bcl-2 expression was performed with the Microsoft Excel program.

**Results:** Bcl-2 expression is shown in Fig. 1. In women without endometriosis, a low level of Bcl-2 expression was seen for endometrium in the early-proliferative and late secretory phases. High levels of Bcl-2 expression were seen for endometrium in mid-, late-proliferative phases, and early and mid-secretory phases. There was a significant negative correlation between Bcl-2 expression and spontaneous apoptosis in endometrium from controls ( $r = -0.473$ ,  $P = 0.035$ ). In women with endometriosis, Bcl-2 expression was high in all stages of proliferative phase endometrium but was undetectable or barely detectable in early- and mid-secretory phase endometrium. In late-secretory phase endometrium, Bcl-2 expression was detectable in 5/6 samples but was highly variable. Furthermore, there was no correlation between Bcl-2 expression and apoptosis ( $r = 0.129$ ,  $P = 0.49$ ) in endometrium from women with endometriosis.



**Fig.1 Bcl-2 Expression In Uterine Endometrium**

**Conclusions:** These results suggest that the normal control of apoptosis in uterine endometrium is disrupted in women with endometriosis.

#### P-116

**Apoptosis and expression of Mcl-1, Bcl-2, and Bax in human endometriotic tissues following gonadotropin releasing hormone analog treatment.** H. Chen, H. Au, T. Sheen, R. Wang, P. Yang, C. Tzeng. Ctr for Reproductive Medicine, Dept of Obstetrics and Gynecology, Taipei Medical Univ Hosp, Taipei, Taiwan.

**Objective:** The gonadotropin-releasing hormone analog (GnRHa), leuplin depot, has been widely used in the treatment of endometriosis. The action of GnRHa on endometriosis has been proven to result in down regulation of pituitary GnRH secretion, but also has a direct effect on endometriotic tissues. In this study, apoptosis and the expression of Mcl-1, Bcl-2, and Bax were examined in normal endometrium and endometriotic tissues both before and after GnRH analog (GnRHa) treatment.

**Design:** prospective control study in a laboratory of a university hospital.

**Materials/Methods:** Women who were undergoing either diagnostic laparoscopy for endometriosis, infertility, or tubal ligation were included in this study. Twenty normal endometrial samples during the menstrual cycle and 10 chocolate cyst samples with endometriosis before or after GnRHa (leuprolide)-treatment were collected, formalin-fixed, and paraffin-embedded. Apoptotic cells were detected using the terminal dUTP nick-end labeling (TUNEL) assay for DNA fragmentation; Mcl-1, Bcl-2, and Bax protein expression were demonstrated with immunohistochemical technique.

**Results:** Apoptotic cells were rare in the eutopic endometrium (chocolate cyst wall) of women with endometriosis (8.2%). In contrast, women with endometriosis showed significantly increasing numbers of apoptotic cells after GnRHa treatment (57.8%;  $P < 0.05$ ). The anti-apoptotic protein, Mcl-1, was positive in all tissues from cases in the early secretory phase