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PCOS

Risk of Uterine Leiomyomata

Polycystic ovary syndrome (PCOS) correlates positively with uterine leiomyomata (UL) in African-American women, according to researchers in the United States.

Women with PCOS are known to be at higher risk for infertility, endometrial carcinoma, insulin resistance, diabetes, hypertension, and cardiovascular disease (Rebuffe-Scrive et al., 1989; Dahlgren et al., 1991, 1992; Dahlgren & Janson, 1993). It is not known, however, if these women have a higher risk for uterine leiomyomata. It is notable that some studies have reported a link between UL risk and medication-treated diabetes (Faerstein et al., 2001) and hypertension (Faerstein et al., 2001; Boynton-Jarrett et al., 2005).

L.A. Wise and colleagues carried out this study to evaluate the relationship between PCOS and UL risk. "The present study suggests a positive association between PCOS and UL in African-American women," wrote Wise et al. ("Polycystic Ovary Syndrome and Risk of Uterine Leiomyomata," *Fer & Ster*, 2007;87(5):1108-1115).

The study included 23,571 premenopausal women with no history of UL at study entry; all were participants in the Black Women's Health Study, which studied African-American women 21-69 years old in 1995 who lived in the United States (Rosenberg et al., 1995). The women completed mailed questionnaires regarding health status every two years. Women reported polycystic ovary syndrome or Stein-Leventhal syndrome under an open-ended question regarding "other serious illness" on the 1995 and 1997 questionnaires; follow-up questionnaires asked specifically about diagnoses of polycystic ovarian syndrome by a physician. The reported year of PCOS diagnosis had to precede that of UL diagnosis, as this was a prospective analysis. Data were also obtained on age at menarche, oral contraceptive use, height, weight, BMI, parity, weight at 18 years of age, physician-diagnosed diabetes, alcohol intake, cigarette smoking, education, occupation, and history of infertility. The accuracy of self-report was assessed in a random sample of 248 cases.

Analysis included Cox regression models and estimation of incidence rate ratios (IRRs) and 95% confidence intervals (CIs). $P < 0.05$ was considered significant and all analyses utilized SAS statistical software (SAS Institute, 2002).

Prevalence of PCOS at the end of follow-up was 1.2% (n=275). During 114,373 person-years of follow-up, 3,631 cases of UL were confirmed either by ultrasound (n=2,926) or hysterectomy (n=705). The incidence of UL was 65% higher among PCOS women (fully adjusted IRR, 1.65; 95% CI, 1.21-2.24).

IRRs stayed elevated with increasing time after PCOS diagnosis. Results were similar for analysis that was restricted to women who reported recent PAP smears (IRR for women with PCOS vs. without PCOS, 1.64; 95% CI, 1.20-2.24).

The authors concluded, "In the present study of African-American women, PCOS was associated with a 65% increase in the risk of UL."

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Insulin Resistance

Increased Incidence of Spontaneous Abortion Following ART

Insulin resistance is associated with an increased incidence of spontaneous abortion, state researchers in Australia.

A recent study reported that overweight and obese infertile women have a high rate of spontaneous abortion following assisted reproductive technology (ART) (Wang et al., 2002). However, other authors disagree with this conclusion (Styne-Gross et al., 2005). Polycystic ovary syndrome (PCOS) may be part of this confusion because it has been listed as a risk factor for spontaneous abortion, most likely related to obesity (Hamilton-Fairley et al., 1992). Nevertheless, this may be the result of the high incidence of overweight/obesity in women with PCOS (Wang et al., 2001). Despite these studies, there is no obvious mechanism suggested for the association.

Due to these conflicting results, Li Tian and colleagues conducted a study to determine the influence of IR on the risk of spontaneous abortion in women undergoing ART ("Insulin Resistance Increases the Risk of Spontaneous Abortion after Assisted Reproduction Technology Treatment," *The Journal of Clinical Endocrinology & Metabolism*, April 2007;92(4):1430-1433). "This study suggested that IR was an independent risk factor for spontaneous abortion," wrote Tian et al.

This study included 107 patients who achieved their first pregnancy following ART in a tertiary medical center. A homeostasis model assessment of IR (HOMA-IR) was performed. The patients' demographic characteristics and pregnancy results were documented, as well. Statistical assessment was made of patients with and without IR. Logistical regression analysis was used to evaluate the influence of IR as well as a number of other factors simultaneously as to the risk of spontaneous abortion.

The rate of spontaneous abortion was 17.8%. Following adjustment for other risk factors, a strong association was noted between IR and the risk of spontaneous abortion. The influence of overweight/obesity and PCOS showed no statistical significance in the multivariate model.

"Because of the high prevalence of IR in obese or polycystic ovarian syndrome patients, the risk of spontaneous abortion in these patients can be raised," wrote Tian et al. "Patients with IR should be advised to improve their insulin sensitivity through lifestyle changes or medical intervention before infertility treatment to reduce their risk of spontaneous abortion."

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Oligozoospermia**Seminal Adrenomedullin Immunoreactivity Levels Correlate with Semen Biochemical Parameters**

Patients with oligozoospermia have higher levels of seminal adrenomedullin (AM) immunoreactivity, state researchers in Italy, and these levels correlate with semen biochemical parameters.

AM has been detected in both human and rat pituitary (Montuega et al., 2000), as well as in ovarian and follicular fluid (Giacalone et al., 2003; Marinoni et al., 2002). AM and its receptor have been detected in human granulosa lutein cells at the preovulatory stage and the midluteal phase, demonstrating that AM is a local factor enhancing progesterone production by these cells (Moriyama et al., 2000). AM has also been found in human testis (Santemma et al., 2001) and prostate (Jimenez et al., 1999), and the large amounts of AM detected in human seminal fluid reportedly correlate with sperm mobility (Marinoni et al., 2005).

The goal of this study by E. Marinoni and colleagues was to evaluate the role of AM in male fertility. "We conclude that in human seminal fluid AM concentration is increased in infertile oligozoospermic patients and derives very likely from the prostate. Its role in the regulation of male fertility, however, has to be understood," wrote Marinoni et al. ("The Level of Adrenomedullin Immunoreactivity in Seminal Fluid is Higher in Oligozoospermic Subjects and Correlates with Semen Biochemical Parameters," *EJOG*, 2007;131:169-175).

Included in the study were 51 healthy males who were 25-41 years old and attending an infertility clinic due to male factor, female factor, or both. Ejaculate was retrieved and analyzed according to WHO (1999) standards; subjects were then divided into normospermic (n=19), oligozoospermic (n=17), and azoospermic (n=15; 7 obstructive and 8 non-obstructive). Blood samples were collected at the time of sperm collection and serum LH, FSH, prolactin, and testosterone were measured by commercial immunoassay. AM was assayed in seminal plasma and blood samples as described previously (Di Iorio et al., 1998). Sperm count, motility, morphology, and trajectory characteristics were recorded, and levels of fructosio, citric acid, L-carnitine, and nitric oxide were determined.

Hormonal and AM concentrations are given as mean \pm SEM. Statistical analysis included Kruskal-Wallis one-way analysis of variance, determination of Spearman rank order correlation, and stepwise linear regression; $P < 0.05$ was considered significant.

Seminal plasma AM concentrations were 305 ± 30 pg/ml in oligozoospermic men, versus 209 ± 10 pg/ml in normospermic men ($P < 0.05$). AM levels in semen were 155 ± 25 pg/ml in patients with nonobstructive azoospermia, which was significantly ($P < 0.05$) less than that found in patients with obstructive azoospermia (252 ± 27 pg/ml).

Significant correlations were noted between plasma AM and prolactin (correlation coefficient=0.724; $P < 0.01$) and percentage of motile sperm (correlation coefficient=0.528; $P < 0.05$). No significant relationships were noted between AM and any other semen parameters or hormone levels.

Circulating AM correlated significantly ($P < 0.05$) with prolactin levels in normospermic (correlation coefficient=0.714), oligozoospermic (correlation coefficient=0.797), and obstructive azoospermic patients (correlation coefficient=0.790).

Significant ($P < 0.01$) correlations were noted between seminal plasma AM and citric acid levels in oligozoospermic (correlation coefficient=-0.679) and obstructive azoospermic men (correlation coefficient=1.000).

Seminal plasma levels of AM did not correlate with any semen biochemical parameters or plasma hormone concentrations in normospermic controls.

"In summary, our study confirms previous observations that high levels of AM are present in human seminal fluid and in fertile males correlates with sperm motility. We found higher levels of seminal plasma AM in oligozoospermic subjects, which correlates with prostate marker, and a reduced AM concentration in secretory azoospermia, suggesting that AM may

play a role in seminal fluid function, although further extensive studies are necessary for a better understanding of the mechanisms involved," stated Marinoni et al.

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ART

Silver-Russell Syndrome in Girl Born after In Vitro Fertilization

Hypermethylation of *PEG1/MEST* may correspond with low birthweight (LBW) in assisted reproductive technology (ART) conceptions with Silver-Russell syndrome (SRS), state researchers in Japan.

The gene responsible for SRS has not been identified, but candidate regions are 7p11.2-p13 (Monk et al., 2000) and 7q31-qter (Hannula et al., 2001), and candidate genes include *GRB10* on 7p12 and *PEG1/MEST* (Hitchins et al., 2001). In addition, studies have shown hypomethylation of the differentially methylated region (DMR) located upstream of *H19* (*H19*-DMR) in a large proportion of SRS patients (Gicquel et al., 2005).

M. Kagami and associates conducted this study to examine methylation pattern in a girl conceived by ART who had SRS. "The results suggest that hypermethylation of paternally expressed genes including *PEG1/MEST*, which usually have growth-promoting effects, may be relevant to LBW in subjects conceived by ART," wrote Kagami and colleagues ("Silver-Russell Syndrome in a Girl Born after In Vitro Fertilization: Partial Hypermethylation at the Differentially Methylated Region of *PEG1/MEST*," *J As Rep Gen*, 2007;24:131-136).

The subject of this study, a Japanese girl, and her twin sister were conceived with IVF and delivered by cesarean section at 37 weeks; the subject was seen at 3 6/12 years of age due to short stature. Her twin sister was clinically normal. Physical examination revealed SRS-compatible features in the shorter twin.

Methylation pattern was analyzed for the DMR of *PEG1/MEST* using leukocyte genomic DNA of the twins and parents. A genomic sequence containing 31 CpG dinucleotides was amplified by polymerase chain reaction (PCR) with a methylated allele specific primer pair (MET) hybridizing to a region that contained unconverted methylated cytosines and an unmethylated allele specific primer pair (UNMET) hybridizing to a region that contained thymines converted from unmethylated cytosines (Kosaki et al., 2000). PCR products were then subjected to direct sequencing. Sequencing analysis was also performed with another primer pair (COMMON) that hybridizes to a region lacking CpG dinucleotides (Kobayashi et al., 2001). DNA samples of a previously described SRS patient with mUPD7 (Kosaki et al., 2000) and 50 normal subjects were examined as controls. Methylation analysis was performed for the *H19*-DMR, as well. In addition, methylation status was analyzed for a genomic sequence containing 10 CpG dinucleotides within the DMR of small nuclear ribonucleoprotein polypeptide N (*SNRPN*) on 15q11.2. Microsatellite analysis was conducted for 14 loci widely dispersed on chromosome 7.

Eight of 31 cytosines in the patient, and four of 31 in the father, were cytosines, not thymines, in the PCR products amplified with UNMET. In the mother, sister, and 50 controls, all 31 cytosines at the CpG dinucleotides were delineated as cytosines and thymines in the PCR products amplified with MET and UNMET, respectively.

PCR products were obtained only with MET in the SRS patient with mUPD7, and all 31 cytosines at the CpG dinucleotides were detected as cytosines. Sequencing analysis using COMMON confirmed results and showed the absence of abnormal methylation in the MET and UNMET hybridizing region.

In the patient, methylated and unmethylated alleles of *H19*-DMR occurred at similar frequencies and methylation pattern of the DMR at *SNRPN* was normal.

Genotyping results were consistent with biparental origin of chromosome 7 in the patient and sister. For six of the seven loci that were examined in the sister, the twins inherited different alleles

from the father and the same allele from the mother. Paternal origin of the alleles for *D7S515* and *D7S684* flanking *PEG1/MEST* differed between the girls.

"In summary, we observed partial hypermethylation in an SRS girl conceived with use of ART. Further studies will serve to clarify the relevance of abnormal imprinting to the high prevalence of LBW with and without SERS phenotype in subjects born after ART," wrote Kagami et al.

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Semen Parameters

Correlation between SABP Expression and Semen Quality

When spermatozoan expression of secretory actin-binding protein (SABP) is higher, semen quality is lower, state researchers in the Czech Republic.

SABP is a glycoprotein that was originally isolated from human seminal plasma; its interaction with actin was detected by agar gel immunoelectrophoresis by Akyama and Kimura (1990). Also known as gp17 factor, SABP has the ability to bind CD4 surface marker of T cells, macrophages and sperm cells (Autiero et al., 1991). Several studies have proposed that gp17 may prevent interaction of other molecules with CD4, thereby protecting the sperm surface in the male and female genital tracts (Autiero et al., 1991, 1995, 1997; Bergamo et al., 1997; Caputo et al., 1998, 1999).

In the current study, J. Capkova and associates sought to determine the specificity of Hs-16 monoclonal antibody (mAb) and localization and frequency of Hs-16-recognized protein on human spermatozoa. "Hs-16 antibody reacts specifically with SABP. SABP can serve as a marker of defective sperm and may be associated with fertility failure," wrote Capkova et al. ("Increased Expression of Secretory Actin-Binding Protein on Human Spermatozoa is Associated with Poor Semen Quality," *Hum Rep*, 2007;22(5):1396-1404).

The authors studied sperm samples from 30 fertile men with normal spermograms and 30 men with pathological spermograms. The western blotting technique, as well as matrix-assisted laser desorption/ionization mass spectrometry was used to determine the specificity of Hs-16 mAb. Sperm ejaculates were then tested with indirect immunofluorescence with Hs-16 antibody.

Statistical analysis included one-way analysis of variance and Student-Newman-Keuls' test; $P < 0.01$ was considered significant for the former, $P < 0.05$ was significant for the latter.

Hs-16 mAb reaction patterns were the same with human spermatozoa and seminal plasma and were found to be specific to human spermatozoa.

The frequency of staining varied when spermatozoa were labeled with anti-actin antibody and Hs-16 mAb; anti-actin antibody stained spermatozoa at a higher frequency than did Hs-16 mAb. Localization of Hs-16-detected protein (SABP) was not uniform on the sperm surface; 70% of labeled cells had staining on the midpiece and sperm tail, while 30% of labeled cells had staining on other parts and the staining was weaker.

SABP expression was significantly higher for asthenozoospermic (mean value 19.8%; $P < 0.01$) and oligoasthenoteratozoospermic (mean 28.9%; $P < 0.01$) men compared to those with normal spermograms (mean 8.96%). Men with oligoasthenoteratozoospermia had significantly ($P < 0.05$) higher SABP expression than did those with asthenozoospermia.

In conclusion, Capkova and colleagues stated, "We have assessed 60 semen samples from different donors and we observed significantly higher expression of Hs-16-detected protein on spermatozoa from asthenozoospermic and/or oligoasthenoteratozoospermic donors, in comparison with normozoospermic samples. Nevertheless, there were six samples in the normozoospermic group with relatively high Hs-16 expression ($\approx 15\%$ of labeled cells). Such samples might fail in fertilization."

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IVF

Effect of Embryo Replacement Depth on Outcome

The depth at which embryos are placed affects the outcome of in vitro fertilization (IVF), states a report from Italy.

Research has shown that embryos should be placed in the uterus with as little trauma as possible (Hearns-Stokes et al., 2000; Matorras et al., 2002; Yaniv et al., 2001). Successful embryo transfer requires that the endometrium and uterine fundus not be touched when the embryos are replaced in the lumen of the endometrial cavity (Kovasc, 1999; Salha et al., 2001). Traditionally, the embryo is placed ≈ 10 mm below the fundal endometrial surface (Leeton et al., 1982); however, some researchers have stated that placing the embryos lower may improve the pregnancy rate (Wood et al., 2000).

This study by A. Pacchiarotti and colleagues aimed to determine how the depth of embryo transfer placement affects clinical pregnancy rate. "The results suggest that the depth of embryo replacement may be an important variable in embryo transfer technique," wrote Pacchiarotti et al. ("The Impact of the Depth of Embryo Replacement on IVF Outcome," *J As Rep Gen*, 2007;24:189-193).

The authors collected data from 104 consecutive embryo transfers performed on 104 women who were 26-37 years old. Standard down-regulation protocol was followed for ovarian stimulation, and transvaginal ultrasound-guided oocyte retrieval was carried out 36 hours after hCG administration. Ultrasound-guided embryo transfer was conducted 48 hours after insemination. Patients were randomly assigned to two study groups according to the distance between the tip of the catheter and the uterine fundus: group A, $> 10 < 15$ mm; group B, ≤ 10 mm. Transfer was performed by the same provider and the same transfer technique was used for all patients.

Statistical analysis included χ^2 test and Student's t-test, with $P=0.05$ considered significant. Data are given as mean \pm SD.

Embryo transfer characteristics, demographic characteristics, and main cycle parameters did not differ significantly between groups.

The amount of FSH required for groups A and B did not differ significantly (3780 ± 543 IU vs. 3633 ± 868 IU, respectively), nor did estradiol levels on the day of HCG administration (1897 ± 761 vs. 1902 ± 752), mean number of oocytes retrieved (8.2 ± 2.4 vs. 7.2 ± 2.1), and mean number of embryos transferred (2.1 ± 1.2 vs. 1.8 ± 1.6).

Pregnancy rates were significantly higher in group A (27.7%) compared to group B (14% ($P=0.009$), as were implantation rates (15.3% vs. 7.1%, respectively; $P=0.027$). Abortion and ectopic pregnancy rates did not differ significantly between A and B.

"In conclusion, maybe the depth of the embryo replacement should be important to improve success of implantation rate, though more studies are warranted to confirm our results," stated Pacchiarotti et al.

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Testicular Sperm Extraction

Relevance of Tissue Perfusion

The quality and quantity of sperm in azoospermic patients is dependent upon the intensity of tissue perfusion, according to an Austrian study.

While testicular sperm extraction (TESE) has proven reliable and successful for sperm recovery in male factor-dependent infertile couples (Kahraman et al., 1996; Palermo et al., 1999; Sukcharoen et al., 2001), it remains difficult to predict which azoospermic patients may have sperm present for surgical sperm retrieval (Wood et al., 2002). Studies have recently proposed that testicular tissue

perfusion be used to improve the chances of sperm retrieval from testicular biopsy (Herwig et al., 2004a, b).

Further research by R. Herwig and colleagues in the current study sought to determine the relationship between the level of testicular tissue perfusion and quantity and quality of sperm recovered from these areas. "We have shown for the first time that in patients suffering from azoospermia, sperm quality and quantity depend on tissue perfusion within the testicle," stated Herwig et al. ("Tissue Perfusion-Controlled Guided Biopsies are Essential for the Outcome of Testicular Sperm Extraction," *Fer & Ster*, 2007;87(5):1071-1076).

Included in the study were 12 azoospermic patients undergoing TESE biopsy (Herwig et al., 2004a, b) for sperm retrieval in an assisted reproduction program. Forty biopsies were taken from 20 testicles (10 right side and 10 left side). Four patients had only one testicle due to testicle ablation for treatment of testicular tumors. Preoperatively, testicular perfusion mapping was carried out using contrast-enhanced, high-resolution color Doppler ultrasound and repeated intraoperatively. A 22-gauge needle was placed in areas with the best perfusion, and a small incision was made with radiofrequency cutting. Exposed tissue was screened with a laser Doppler probe and perfusion rates were then determined using a laser Doppler flowmeter. Tissue perfusion units (TPU) were defined arbitrarily based on mean cell velocity and average concentration of moving blood (mL/min/100g). Random biopsies were taken from the same testicle and TPU was measured for these samples, as well (Herwig et al., 2004; Schuster et al., 2004). Testicular biopsy-score counts (Johnsen score) were taken from random biopsies (Johnsen, 1970). Sperm morphology was assessed according to WHO (2006) standards. Motile sperm with normal morphology were used for ICSI.

Statistical analysis included Student's t-test and Pearson correlation coefficient calculation; $P < 0.05$ was considered significant. Data are given as means and SD.

The authors were able to find sperm in all 12 patients; 9 of 12 had motile sperm with apparently normal morphology that was suitable for ICSI, but 3 of 12 had no normal sperm.

TPU levels were higher in perfusion-controlled biopsies, and more normal sperm were found with compared with randomly-obtained samples. Sperm with higher quality and quantity were found in areas with high tissue perfusion ($0.85, P=0.001$).

Areas with >70 TPU had 74.42% progressive A quality sperm, but areas with ≈ 40 TPU had $65.94\% \pm 4.93\%$ motile sperm, and those with 20-40 TPU had an even lower number of motile sperm at $48.84\% \pm 6.02\%$. Areas that had ≈ 10 TPU had only $2.75\% \pm 4.7\%$ normal sperm, with the majority being abnormal and precursor cells.

The fertilization rate using motile sperm with apparently normal morphology for ICSI was 76 to 89%. No significant difference was noted in fertilization rate with use of sperm from different TPU level areas.

"We documented for the first time that the level of tissue perfusion matches well with the level of recovered sperm in both quality and quantity," concluded Herwig and colleagues. "Our novel technique of perfusion-controlled testicular biopsy allows for predictable TESE, and better sperm samples for ICSI and cryopreservation in assisted reproduction. We therefore recommend this new method of sperm retrieval in azoospermic patients."

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PCOS

Adrenal Androgen Excess has Genetic Basis

There is a strong correlation between adrenocorticotrophic hormone-stimulated steroid levels in women with polycystic ovary syndrome (PCOS) and their sisters, which supports a genetic origin of the adrenal androgen excess found in PCOS, state researchers in the United States.

PCOS is a common hyperandrogenic disorder that occurs in approximately seven percent of women of reproductive age (Knochenhauer et al., 1998); it is associated with menstrual

disturbances, polycystic ovaries, hirsutism as well as increased incidence of infertility, diabetes mellitus, and cardiovascular disease. Numerous diagnostic criteria have been suggested for PCOS; however, hyperandrogenism is a common feature of all of them (Revised 2003 Consensus on Diagnostic Criteria and Long-Term Health Risks Related to PCOS). Adrenocortical dysfunction and adrenal androgen (AA) excess may result from inherited or acquired factors. Studies in siblings of patients with PCOS indicate that AA levels, as evidenced by levels of the AA metabolite circulating dehydroepiandrosterone sulfate (DHEAS), for the most part reflect the influence of genetic factors (Azziz et al., 2006; Legro et al., 2002).

Based on the above findings, Mark O. Goodarzi, MD, PhD and colleagues conducted a study in which they tested the hypothesis that adrenocortical biosynthesis in PCOS is strongly heritable ("Correlation of Adrenocorticotropin Steroid Levels between Women with Polycystic Ovary Syndrome and their Sisters," *American Journal of Obstetrics & Gynecology*, April 2007;196:398.e1-398.e6). "Women with polycystic ovary syndrome and their sisters have significantly correlated levels of adrenocorticotropic hormone-stimulated steroids, supporting a genetic basis of the adrenal androgen excess observed in polycystic ovary syndrome," wrote the researchers.

An adrenocorticotropic hormone stimulation test was performed in 27 women with PCOS and 28 of their sisters. Sixty minutes later, the steroids dehydroepiandrosterone, androstenedione, and cortisol were measured; these values were used to analyze sister-sister association by regression analysis.

A significant association was observed in the adrenocorticotropic hormone-stimulated (60-minute) log-transformed dehydroepiandrosterone and cortisol figures between sisters ($r=0.47$, $P=0.01$ and $r=0.57$, $P=0.01$, respectively). A similar trend was noted in the 60-minute A4 values ($r=0.29$, $P=0.06$).

"Our data support the hypothesis that adrenocortical steroidogenesis is under significant genetic control and likely reflects variations in the genes controlling their biosynthesis and/or metabolism," stated Goodarzi et al.

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ART

Pregnancy Achieved in Patient with Severe Congenital Bicuspid Aortic Valve Stenosis

Researchers in Turkey present a case in which a woman with severe congenital bicuspid aortic valve stenosis achieved pregnancy with assisted reproductive technology (ART).

Aortic stenosis in women occurs, for the most part, because of rheumatic heart disease and congenital bicuspid aortic valve. Disease-related complications may arise due to the increased preload during pregnancy; these complications include arrhythmia, congestive heart failure, and myocardial ischemia (Sobelga et al., 2004).

The current study by C.T. Erel and colleagues present a woman with a severe bicuspid aortic valve who had a twin pregnancy after intracytoplasmic sperm injection (ICSI) and embryo transfer (ET). "A woman with a severe congenital bicuspid aortic valve may get pregnant and deliver healthy newborns with intensive prenatal care and follow-up," wrote Erel et al. "The severe congenital bicuspid aortic valve stenosis may not be considered an absolute contraindication for assisted reproductive techniques and pregnancy" ("Successful Pregnancy Achieved by Assisted Reproductive Techniques in a Woman with Severe Congenital Bicuspid Aortic Valve Stenosis," *Arch Gyn Obstet*, 2007;275:299-300).

The 38-year-old woman presented with an eight-year history of infertility due to male factor. She was previously diagnosed with congenital bicuspid aortic valve and was not symptomatic. Her bicuspid aortic valve had an area of 0.4 cm^2 , peak aortic gradient of 57 mmHg, and ejection fraction of 67%.

Only two embryos were transferred in the first ICSI attempt to limit the risk of obstetric complications. A twin pregnancy was achieved; the patient was informed about the possible complications during pregnancy and the risk of severe prematurity. The pregnancy progressed with no cardiac complications, however, and no significant changes were noted on echocardiography parameters at the 24th gestational week.

Fetal echocardiograms showed normal heart structure and function for both fetuses. At the 36th week of gestation, an emergency cesarean section was performed due to fetal distress. Two healthy babies were delivered, and prophylactic antibiotics were given to prevent infective endocarditis. The patient was discharged four days after cesarean with no complications.

"Women with severe congenital bicuspid aortic valve who have fertility desire should be informed about the pregnancy-related complications, surgical cardiac interventions during pregnancy and the possibility of neonatal cardiac defects. Echocardiography investigation is essential for the assessment of severity of the disease. Close cardiologic follow-up during pregnancy is the crucial component of an appropriate antenatal care, both for the mother and the fetus," concluded the authors.

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IVF

Antral Follicle Count is a Good Indicator of Ovarian Response

Antral follicle count (AFC) provides a good indication of ovarian response for in vitro fertilization (IVF) patients, state researchers in the Netherlands.

Several studies have shown that ovarian volume, as observed by transvaginal ultrasonography, is a good predictor of ovarian reserve (Syrop et al., 1995; Tomas et al., 1997; Bansci et al., 2004). It has also been confirmed that a decrease in ovarian volume is an early indicator of depleted follicles and its measurement is most likely to be of value (Lass et al., 1997). Endocrine tests have been compared as predictors of total number of follicles obtained following stimulation (Kwee et al., 2003). Research has been conducted to find a simple, single test to be used to identify poor, normal and hyper responders (Kwee et al., 2006).

In a study aimed at predicting poor and hyper responders, Janet Kwee and colleagues compared AFC and basal ovarian volume (BOV), the exogenous FSH ovarian reserve test (E.F.O.R.T.) and the clomiphene citrate challenge test (CCCT) ("Ovarian Volume and Antral Follicle Count for the Prediction of Low and Hyper Responders with In Vitro Fertilization," *Reproductive Biology and Endocrinology*, 2007;5(9):1-10). "AFC is able to accurately predict the number of follicles obtained during maximal ovarian stimulation," wrote the authors.

This prospective, randomized study included 110 regularly menstruating women, ages 18-39 years. The participants were divided into two groups: 56 patients underwent a CCCT and 54 patients underwent an E.F.O.R.T. A transvaginal ultrasonography was performed on all patients to measure the BOV and count of basal antral follicle. A standard IVF treatment was performed on all patients following the test.

The best indicator of ovarian reserve (Y) was observed in a multiple regression model, which included AFC, inhibin B-increment in the E.F.O.R.T. and BOV concurrently [$Y = -3.161 + 0.805 \times \text{AFC} (0.258-1.352) + 0.034 \times \text{Inh. B-incr.} (0.007-0.601) + 0.511 \text{ BOV} (0.480-0.974)$ ($r = 0.848$, $P < 0.001$).

Univariate regression revealed that the most accurate indicators for poor response were the CCT (ROC-AUC = 0.87), the bFSH (ROC-AUC = 0.83) and the AFC (ROC-AUC = 0.83). Multiple logistic regression analysis failed to produce a superior model in regards to improving the prediction of poor response.

Univariate logistic regression revealed that the most accurate predictors for hyper response were AFC (ROC-AUC = 0.92) and the inhibin B-increment in the E.F.O.R.T. (ROC-AUC = 0.92), however, AFC had more suitable test characteristics, specifically a sensitivity of 82% and a

specificity of 89%. Multiple logistic regression analysis failed to produce a superior model in regards to the prediction of hyper response.

"In conclusion AFC performs well as a test for ovarian response being superior or at least similar to complex expensive and time consuming endocrine tests," reported Kwee et al. "It is therefore likely to be the test for general practice."

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PCOS

Upper-Body Obesity Intensifies Insulin Resistance and Hyperandrogenism

Upper-body obesity in patients with polycystic ovary syndrome (PCOS) exacerbates insulin resistance and hyperandrogenism, and alters β -cell function, states a researcher in Iraq.

PCOS is the most frequently occurring type of anovulatory infertility in reproductive-age women. Its symptoms include hyperandrogenemia, chronic anovulation, and decreased fertility (Balen et al., 1995). Women with PCOS have an increased incidence of obesity, insulin resistance, type II diabetes, and premature arteriosclerosis. Obesity is seen in 35-60% of women with PCOS (Balen et al., 1995). PCOS patients have an increased incidence of abnormal β -cell function (Campelli and Lanzore, 1998; Mark et al., 2005). Upper-body obesity is strongly associated with cardiovascular disease and type II diabetes, while lower-body obesity is not.

Based on the above information, Ansam A. Al-Bayatti, M.B., Ch.B., M.Sc., Ph.D. conducted a study to evaluate the association between insulin resistance and β -cell function in PCOS patients with upper-body obesity ("Insulin Resistance and Upper-Body Obesity in Polycystic Ovary Syndrome," *Middle East Fertility Society Journal*, 2006;11(3):202-209). "Upper-body obesity aggravates insulin resistance and hyperandrogenism of patients with PCOS and modulates β -cell function," reported Al-Bayatti.

Fifty-one Iraqi PCOS patients and 25 healthy age-matched women serving as controls were included in this cross-sectional study. The PCOS women were divided into two groups based on waist-to-hip ratio (WHR): >0.85 (upper-body obesity) or ≤ 0.85 (lower-body obesity). Levels of fasting insulin, glucose, and free testosterone (free T) were assessed. Homeostatic model assessment values of IR (HOMA-IR) and the percent of β -cell function (HOMA-% β -cell) were evaluated. The student-t test, analysis of variance (ANOVA), and the Pearson correlation coefficient (r) were used for statistical analysis where suitable.

Sixty percent of obese PCOS patients had upper-body obesity and were more insulin resistant and had higher β -cell function than PCOS patients with lower-body obesity who, in turn, were more insulin resistant than the controls ($P < 0.0005$ by ANOVA).

In the PCOS patients, upper-body obesity was associated positively and significantly with HOMA-IR, HOMA-% β -cell, and free T ($r=0.371$, $P=0.002$; $r=0.383$, $P=0.001$; $r=0.254$, $P=0.027$, respectively). In the PCOS group, 76.5% had IR; 65% of these had upper-body obesity.

"In conclusion, patients with high PCOS have 76.5% prevalence of insulin resistance, upper body obesity aggravates insulin resistance and hyperandrogenism of patients with PCOS and modulate β -cell function," wrote the author.

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Fertility Weekly

Conference Abstracts . . . May 21, 2007

Information About Research Worldwide . . . Compiled by June Temples

"A Nurse Intervention Model in the Fertility Preservation Clinic for Female Cancer Patients." *A. Alon, D. Kovalski, R. Kapustiansky, R. Homburg, A. Amit, and F. Azem. Tel Aviv Sourasky Medical Center, IVF Unit, Tel Aviv, Israel.*

According to an abstract presented by the authors at the 22nd Annual Meeting of the European Society of Human Reproduction and Embryology in Prague, Czech Republic, June 18-21, 2006, "**Introduction:** The principles of cancer treatment now lie in 'quality survival'. Anticipating the ovarian damage from chemotherapy, fertility preservation can improve the quality of life for cancer patients after recovery, providing hope for a future pregnancy. Fertility preservation for female cancer patients has become an essential consideration owing to substantial improvement of long-term survival and assisted reproductive technologies (ARTs) are now capable of providing hope for fertility preservation. The nurse's role is becoming an integral part of this process. The aim of this paper is to outline an intervention model for nurses providing care for cancer patients in fertility preservation and to emphasize the importance and different stages of the nurse's role. **Materials and methods:** This intervention model has been constructed on the basis of personal experience and interaction in a dedicated unit, established 5 years ago, in a large hospital. It is the first documentation of such a model. **Results:** The intervention process consists of three stages before, during and after chemotherapy treatment: (i) the first crucial encounter is at the Fertility Preservation Clinic after cancer has been diagnosed and reproductive function has become a significant concern and needs to be considered urgently. The nurse provides full information, arranges documentation and preparation and establishes a rapport with the patient. (ii) The role of the nurse is to accompany and support the crucial decision to proceed and then throughout ART, cryo-preservation and chemotherapy. Coordination of the multidisciplinary units involved is an essential element of this stage. (iii) Follow-up, support and maintaining contact after chemotherapy and, hopefully, up to the stage of *in vitro* fertilization (IVF) to achieve pregnancy. **Conclusions:** This intervention model provides an outline for nurses providing care in fertility preservation and a uniform model that can be used for research."

"Meloxicam Decreases Ovarian Hyperstimulation Syndrome in a Rat Model." *1R. Quintana, 1L. Kopcow, 1M. Diradourian, 1G. Marconi, 2M. Soiza Relly, 1E. Young, and 3D. Paz. 1Instituto Ginecologia y Fertilidad (IFER), Buenos Aires, Argentina; 2Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina; 3Instituto Fisiologia Biologia Molecular y Neurociencias, Cons Nat Invest Cient Tec, Buenos Aires, Argent.*

According to an abstract presented by the authors at the 22nd Annual Meeting of the European Society of Human Reproduction and Embryology in Prague, Czech Republic, June 18-21, 2006, "**Introduction:** Ovarian hyperstimulation syndrome (OHSS) is a complication of ovulation induction. Vascular endothelial growth factor (VEGF) has been considered as a prime causative factor of OHSS progression. A relationship between the enzyme cyclooxygenase-2 (COX-2) and the production of VEGF has been demonstrated in colon and prostate cancers. In the present study we evaluated the effects of Meloxicam (a COX-2 inhibitor) on OHSS-induced rats and compared the results with OHSS-induced rats and with ovulation-induced rats and control group. The objective was to compare ovarian weight, VEGF expression, number of follicles and estradiol levels among groups. **Materials and methods:** Eighty 22 days old female Wistar rats were divided into four groups. Group 1 (control group) (n=20) received 0.1 ml of i.p. saline from day 22 to day 26. Group 2 (mild-stimulated group) (n=20) received 10 IU of PMSG on day 24 and 10 IU of hCG 48 h later (day 26). Group 3 (OHSS group) (n=20) was given 10 IU of PMSG for four consecutive days from day 22 and 30 IU hCG on the fifth day in order to induce OHSS. Group 4

was treated in the same way as Group 3 but receiving 2 μ l (15 mg/ml) of meloxicam 2 h before the PMSG injection, for four consecutive days, and 2 h before the hCG injection on the fifth day. All groups were sacrificed on day 26. The VEGF expression was evaluated by semi-quantitative immunohistochemistry using avidin-biotin peroxidase complex on paraffin sections and Western blotting from homogenates of ovaries. The number of follicles was evaluated on hematoxylin and eosin sections. Ovarian weight was evaluated. **Results:** Hyperstimulation treatment produces a significant increase in the ovarian weight when compared with control animals. There are no differences in the ovary weight between Group 1 and 2 (Group 1: 140 mg+1.8; Group 2: 190 mg+1.4). The OHSS group showed a significant ($p<0.01$) increase in the ovarian weight in relation to control group and mild-stimulated group. This higher weight in the OHSS group was reverted by meloxicam (Group 3: 385 mg+19.6 and Group 4: 235 mg+6.9, $p<0.01$). The number of antral follicles was no different in the four groups but a tendency to a reduction in the number of antral follicles was observed in Group 4 when compared with Group 3. The follicle numbers were as follows: Group 2: 19.8+2.1; Group 3: 41+7 and Group 4: 35.4+11.4. VEGF immuno-reactivity (VEGF-ir) was observed in the theca and stroma cells, with a weakly staining in the granulosa cells of the control group. In the mild-stimulated and OHSS groups, the granulosa cells of pre-ovulatory follicles showed a strong immuno-reactivity with a significant increase in the ovaries from the OHSS group. The ovaries from meloxicam-treated group showed light immuno-reactivity than the OHSS group (*H*-score values are as follows: Group 1: 1.77+0.34; Group 2: 4.05+0.42; Group 3: 7.2+1.44; and Group 4: 4.7+0.52). Western blot showed similar results with a significant difference between hyperstimulated and hyperstimulated+meloxicam ovaries ($p<0.01$). The values (expressed in arbitrary units corrected to the actin concentration) are as follows: Group 3 (OHSS): 11.17+1.28; Group 4 (OHSS+Meloxicam): 6.56+1.97. Serum estradiol levels in hyperstimulated rats showed an increase ~10 times compared with control group. The meloxicam treatment was ineffective in reverting the estrogenic rise in OHSS rats. **Conclusions:** Our results in a rat model suggests that meloxicam may have beneficial effects on OHSS reducing the ovarian weight and VEGF expression."

"Oxytocin Effect on the Control of the Midcycle LH Surge in Women." *P. Vanakara, K. Dafopoulos, A. Kallitsaris, I. Papageorgiou, and I.E. Messinis. University of Thessalia, Larissa, Greece.*

According to an abstract presented by the authors at the 22nd Annual Meeting of the European Society of Human Reproduction and Embryology in Prague, Czech Republic, June 18-21, 2006, **"Introduction:** Previous studies have suggested that oxytocin may participate in the control of midcycle LH peak of the normal menstrual cycle. However, in these studies the LH surge was not fully characterized, since blood samples were taken infrequently. The aim of the present study was to investigate the role of oxytocin in the regulation of LH secretion during the endogenous LH surge of the normal menstrual cycle. **Materials and methods:** Ten healthy normally cycling women volunteered for the study. They were investigated in two cycles, i.e. cycle 1 (control) and cycle 2. Between the two cycles there was a month break. When the diameter of the leading follicle was 16-17 mm, normal saline (cycle 1) or atosiban (cycle 2) was injected i.v. as a bolus followed by infusion for the next 3 h. The dose was 6.75 mg (10 ml) bolus and 417 g/min (2.8 ml/min) for atosiban and 10 ml bolus and 2.8 ml/min for saline. A blood sample was taken from all women before the bolus injection. After the end of the infusion blood samples were taken every 6 h until at least 48 h after the detection of the onset of the endogenous LH surge by urinary LH kits. Serum FSH, LH and oestradiol (E_2) were measured in all blood samples. The area under the curve (AUC) was calculated where appropriate. Statistical analysis was performed by paired *t*-test and repeated measures of one-way ANOVA. The results are expressed as mean SEM. **Results:** All women displayed an endogenous LH surge in both cycles. Follicle size (16.7 \pm 0.08 vs. 16.7 \pm 0.08 mm) and serum E_2 levels (510.1 \pm 73.7 vs. 509.0 \pm 83.3 pmol/l) were similar in the two cycles on the day of the experimental procedure (09:00 a.m.). There were no significant differences in the time interval from the end of the infusion to the onset of the LH surge (46.8 \pm 4.8 h in cycle 1 vs. 45.6 \pm 9.6 h in cycle 2). LH values up to the onset of the surge were similar in the two cycles. The pattern of the LH surge was similar in both cycles. In particular, the duration of the surge was comparable in both cycles (54 1.8 and 61.2 \pm 3.9 h, respectively). Also, the peak value of LH was found to be 22.5 \pm 1.9 h in cycle 1 and 18 \pm 3.1 h in cycle 2 after the surge onset with no difference between the two cycles. However, when individual peak values were considered, significantly lower LH values were found in cycle 2 (49.1 \pm 2.9 IU/l) compared to cycle 1 (62.1 \pm 4.4 IU/l, $p<0.05$). The AUC of

the LH concentrations from the time of the LH surge onset was similar in both cycles (224.2 ± 28.4 vs. 208.9 ± 31.5 IU/l for 54 h). An FSH surge also occurred. FSH values during the whole experimental period were similar in both cycles with no differences in terms of duration of the FSH surge, peak FSH values and interval from onset to peak. The pattern of E_2 changes during the whole experimental period was similar in both cycles. **Conclusions:** The present study demonstrates for the first time that there are no significant effects on blockade of endogenous oxytocin action on the characteristics of the endogenous LH surge. Although individual peak LH values were affected, it is suggested that oxytocin plays only a minor role in the control of pituitary gonadotrophin secretion."

"The Endocrine Cycle Dynamics of Younger Women with Consistently or Variably Elevated Basal FSH Compared to Controls." *1C.H. de Koning, 1J. MacDonnell, 2A.P.N. Themmen, 2F.H. de Jong, and 1C.B. Lambalk. 1Vrije Universiteit Medical Center, Amsterdam, The Netherlands; 2Erasmus Medical Center, Rotterdam, The Netherlands.*

According to an abstract presented by the authors at the 22nd Annual Meeting of the European Society of Human Reproduction and Embryology in Prague, Czech Republic, June 18-21, 2006, **"Introduction:** Elevated early follicular phase FSH is a well-known phenomenon in sub-fertile patients. So far insight in the endocrine mechanism behind this phenomenon comes from studies that compare data from younger vs. older women. Here, we studied the complete endocrine cycle profile of sub-fertile young women with elevated basal FSH compared with controls. Moreover, we distinguished patients with consistently elevated FSH and those with basal FSH levels that fluctuate between elevated and normal. **Materials and methods:** Daily blood samples were taken from 16 controls, 11 patients with elevated early follicular phase FSH in a screening cycle but normal FSH in the study cycle ('High, Low' group) and 11 patients with elevated early follicular phase FSH in both screening and in study cycles ('High, High' group). All subjects had a regular ovulatory cycle. FSH, LH, E_2 , progesterone, inhibin A and inhibin B were measured in the daily serum samples. Anti-Mullerian hormone (AMH) was measured on day 3 in two consecutive cycles for each subject. Statistical analysis was performed by ANOVA, weighted least squares regression models and Chi-squared tests. **Results:** Significantly lower AMH was found both in the 'High, High' and 'High, Low' group compared with the controls. FSH was higher in the 'High, High' group in all phases of the cycle compared with controls and 'High, Low' group. In the early follicular phase and late luteal phase FSH was also higher in 'High, Low' group compared with controls. LH was higher in the 'High, High' group, except for the late follicular and late luteal phase compared with controls. E_2 was lower during the LH surge in the 'High, High' group compared with both 'High, Low' and control groups, but in all other phases E_2 was equal in the three groups. Inhibin A was lower in the early follicular phase in the 'High, High' group compared with controls and higher in the peri-ovulatory phase in the 'High, Low' group. Inhibin B was lower in the 'High, High' group all over the follicular phase compared with 'High, Low' group and controls. 'Normalization' of day 3 FSH in women with previously elevated FSH was associated with inhibin B levels that became normal in the mid-and late-luteal phase of the preceding cycle compared with the lower inhibin B levels when day 3 FSH values remain elevated. **Conclusions:** The persistently low-AMH levels in combination with constantly and intermittently elevated day 3 FSH levels indicates that these younger patients are likely to have diminished ovarian reserve. The endocrine cycle profile in patients with consistently elevated basal FSH resembles in all aspects that of published data from older women. However, patients who present with elevated early follicular phase FSH but normal FSH in the subsequent cycle are characterized by normalization of inhibin B in the preceding luteal phase, indicating a temporary increase of the available cohort. Peri-ovulatory inhibin A hypersecretion in the subsequent cycle could thus be a result of multiple follicle growth."

"A Case of Unilateral Twin Tubal Pregnancy Treated by Methotrexate." *R.S. Karadeniz, S. Dilbaz, S. Deveci, B. Dilbaz, F. Ercan, T. Kinay, and A. Tarcan. Ministry of Health, Etlik Maternity and Women's Health Research and Teaching Hospital, Ankara, Turkey.*

According to an abstract presented by the authors at the 9th Congress of the European Society of Contraception in Istanbul, Turkey, May 3-6, 2006, **"Introduction:** Twin ectopic pregnancy (EP) is an extremely rare entity and among 100 reported cases methotrexate (MTX) treatment has not been reported yet. The incidence of EP is increasing with the wide use of assisted reproductive technologies (ART). Early diagnosis and intervention is important to prevent short and long-

term morbidity. **Case:** A 26-year-old nulliparous woman was admitted to the hospital with a complaint of vaginal bleeding and pelvic pain. She had ovulation induction with gonadotropins and an emergency admission was carried out as there was left adnexial tenderness without acute abdomen signs on pelvic examination. The serum levels of beta-hCG, Hb, WBC, platelet count, ALT and AST were 763 IU/mL, 12 mg/dL, 9060/mm³, 236,000, mm³, 16 and 21 U/L on admission. Two separate tubal pregnancies with a diameter of 17 × 10 and 16 × 10 mm were detected in the left tube during trans-vaginal ultrasonographic examination. There were two predisposing factors for ectopic pregnancy in her history; smoking and a 5-year history of primary infertility. On the third day of hospital admission, multiple dose MTX regimen (1 mg/kg MTX on days 1, 3, 5 and 7, +0.1 mg/kg folic acid on days 2, 4, 6 and 8) was started when beta-hCG level was 987 IU/mL. On the third day an endometrial sampling was performed and endometrium without chorionic villus was reported from pathology department. Labeling the day when the treatment was initiated as Day-1 the serum levels of β-hCG were as follows: on the Day-3=1091 IU/mL, Day-7=750 IU/mL. Color doppler sonography on the third day of treatment showed PI:1.95 and RI:0.78 values in tubal placentation. A single dose of MTX (1 mg/kg) was applied at Day-12 again as the β-HCG level was increased to 1200 IU/mL. Serum β-hCG level was regressed to normal range 32 days after initiation of MTX treatment. **Conclusion:** Patients with unilateral twin tubal pregnancies in literature were managed successfully by operative procedures like; laparoscopic/laparotomic salpingostomy or total salpingectomy. This is pioneering report of a successful MTX use in unilateral twin tubal pregnancy."

"The Efficacy of Interphase-Nuclear-Conversion Induced by Parthenogenetic Activated Fresh Mouse Oocytes." *L. Jiawei Sr., F. Cong, X. Yanwen, Z. Canquan, L. Tao, and Z. Guanglun. The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China.*

According to an abstract presented by the authors at the 62nd Annual Meeting of the American Society for Reproductive Medicine in New Orleans, Louisiana, October 21-25, 2006, "**Objective:** The protocol of interphase-nuclear-conversion induced by fusion of a blastomere with a mouse zygote has been set up as a method of pre-implantation genetic diagnosis (PGD) to detect the chromosomal imbalance in the embryos of translocation carriers. But it was found that metaphase chromosome could not be obtained in some cells. The reasons maybe the fertilization of a mouse oocyte with an abnormal sperm or self activation occurring in aged oocytes, all of which may compromise the cleavage potential of the heterokaryons and then lead to the failure of the conversion. In this study, we used fresh mouse oocytes activated by SrCl₂ to induce the nuclear conversion and compared the efficacy with that of mouse zygotes used. **Design:** A randomized prospective analysis. **Materials and methods:** 227 blastomeres from 61 non-transferred human embryos in our reproductive center were used in the study and divided into two groups randomly, one was fused with mouse zygotes(103 blastomeres, Z-group) and the other with fresh mouse oocytes parthenogenetic activated by SrCl₂(124 blastomeres, P-group). When the nuclear envelop of the heterokaryon disappeared, the cell was fixed and observed under the microscope for chromosome. In the end, the fusion rate, chromosome conversion rate and time required for conversion were compared between these two groups. The use of the non-transferred embryos has got consent from every patient, and the study has been approved by the Ethical Review Board of our hospital. **Results:** 1. There is no significant difference in fusion rate between these two groups (P>0.05).

	Success	Failure	Total	Success Rate (%)
P-group	120	4	124	96.8
Z-group	97	6	103	94.2
Total	217	10	227	

2. The chromosome conversion rate of the P-group is significantly higher than that of the Z-group (P<0.01).

	Success	Failure	Total	Success Rate (%)
P-group	114	61	20	95.0
Z-group	82	15	97	84.5
Total	196	21	217	

3. There is no significant difference between these two groups in the mean time required for chromosome conversion ($P>0.05$), which is 9.27 ± 2.33 hours in P-group and 11.50 ± 5.21 hours in Z-group. But it seems that the range of time is broader in the Z-group. **Conclusion:** There is no significant difference in fusion rate and time required for chromosome conversion between the parthenogenetic activation group and the zygote group, while the chromosome conversion rate is higher in the former group and the range of mean time required is broader in the latter, which implies that parthenogenetic activated fresh mouse oocytes could be a better alternative for induction of premature condensed chromosome, especially for PGD."

"Preimplantation Genetic Diagnosis of Chromosome Rearrangements by Analysis of Metaphase Spreads Obtained After Selective Embryo Biopsy on Day 3." *A. Shkumatov, J. Cieslak-Janzen, V. Kuznyetsov, Y. Ilkevitch, and Y. Verlinsky. Reproductive Genetics Institute, Chicago, Illinois.*

According to an abstract presented by the authors at the 62nd Annual Meeting of the American Society for Reproductive Medicine in New Orleans, Louisiana, October 21-25, 2006, "**Objective:** The purpose of the study was to develop a method to obtain metaphase chromosomes from single blastomeres on Day 3 of embryo development for preimplantation genetic diagnosis (PGD) of chromosome rearrangements. Unlike interphase analysis, which is limited to the availability of locus specific probes that span specific areas of particular chromosomes in order to distinguish balanced and unbalanced embryos, metaphase analysis allows for further differentiation between unbalanced, balanced and normal chromosomal complements. Metaphase chromosomes can be analyzed using commercially available whole chromosome paints and multicolor banding. **Design:** IVF - PGD program. **Materials and methods:** Informed consents were obtained from 21 couples requesting PGD for a chromosome rearrangement. All biopsy procedures were performed on Day 3 with the removal of a single blastomere. Each blastomere removed was chosen based on morphological criteria in the following order: 1st choice - blastomeres without a visible nucleus with clear cytoplasm and 2nd choice - a mononucleate blastomere larger than the other blastomeres for a given embryo. All blastomeres were incubated in Global culture medium supplemented with 10% Plasmanate and 0.1-0.2 $\mu\text{g/ml}$ Colcemid. Those blastomeres exhibiting 1st choice' morphology were fixed after a short incubation of 10 minutes. Those blastomeres with '2nd choice' morphology were observed every hour for interphase-metaphase transition (i.e. nuclear envelope breakdown). Blastomeres were incubated in hypotonic solution for 2-6 minutes prior to fixation with methanol:glacial acetic acid, 3:1. The proportion of metaphase versus interphase nuclei was assessed after having used the morphological selection of the blastomeres and in relation to embryo development. **Results:** From 229 zygotes, 199 (86.9%) had reached 6 or more cells on the morning of Day 3 and considered suitable for biopsy. At the time of biopsy, 20% of embryos had at least one blastomere at metaphase. After an average of 10.2 h in culture 116 (58.3%) blastomeres entered mitosis and were fixed at metaphase versus 83 (41.7%) at interphase. Of 122 biopsied embryos cultured to days 5-6, 64 developed to blastocyst (52.4%). The blastocyst formation rate in biopsied embryos compared to non-biopsied embryos during that same period of time was 52.4% and 48.5% respectively. Embryos in which metaphase chromosomes were obtained more readily gave rise to blastocysts than those at interphase 57% (44 out of 77) versus 44% (20 out of 45) respectively. Analysis of metaphase plates was available for 68.8% of embryos which developed to the blastocyst stage (44 out of 64). Out of 199 blastomeres tested 14 (7%) were found normal and 19 (9.5%) balanced. 4 (19%) patients did not have a transfer due to unbalanced embryos. An average of 1.58 embryos was transferred resulting in a clinical pregnancy rate of 47% (8 out of 17). **Conclusion:** The combination of selective biopsy and incubation in medium with tubulin inhibitors such as Colcemid can ensure high rate of metaphase formation in single biopsied blastomeres. There is a greater potential to obtain metaphase chromosomes for analysis from embryos which reach the blastocyst stage in extended culture (68.8% versus 31.2%). Biopsy of a single blastomere on Day 3 does not adversely affect embryo development."

"The Quantitative and Qualitative Impact of Gonadotropin Stimulation on Human Preimplantation Embryos: A Preliminary Study." *A. Weghofer, S. Munne, W. Brannath, S. Chen, J. Cohen, and N. Gleicher. Yale University Medical School, New Haven, Connecticut; Reprogenetics LLC, West Orange, New Jersey; Vienna University Medical School, Vienna, Austria; Saint Barnabas Medical Center, Livingston, New Jersey; The Center for Human Reproduction, New York, New.*

According to an abstract presented by the authors at the 62nd Annual Meeting of the American Society for Reproductive Medicine in New Orleans, Louisiana, October 21-25, 2006, "**Objective:** Reported aneuploidy rates in infertile women, undergoing ovarian stimulation with gonadotropins, have uniformly been reported as high. Whether these rates are 'natural', or are the consequence of gonadotropin stimulation is, however, unknown. This study, therefore, evaluates the qualitative and quantitative impact of controlled ovarian hyperstimulation with gonadotropins on euploidy rates in human cleavage-stage-embryos. **Design:** Retrospective cohort study. **Materials and methods:** 678 women with normal ovarian function (based on baseline FSH levels <10mIU/ml), at ages 24-47 years, underwent, during IVF cycles, three different stimulation protocols, followed by preimplantation genetic diagnosis (PGD) for chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22. The utilized stimulation protocols involved (1) long agonist stimulation; (2) short agonist stimulation (i.e. a flare or microdose protocol); and (3) antagonist use and were allocated based on stimulation history or other clinical assumptions. Gonadotropin stimulation utilized rec. FSH and/or hMG at varying dosages. Patients underwent PGD for different reasons: Older patients for maternal age, younger patients because of prior implantation failure, patient preference, personal or religious concerns about an abnormal fetus or unwillingness to transfer more embryos. **Results:** Total gonadotropin usage per cycle correlated positively with the absolute number of euploid embryos achieved per IVF cycle ($p=0.03$), but showed a negative correlation with the overall percentage of chromosomally normal embryos ($p=0.02$). LH-containing stimulation resulted in significantly increased percentages of euploid embryos ($p=0.02$), though in the presence of a decreased absolute number of embryos ($p=0.02$). When women were age stratified, the positive effect of LH-including stimulation on euploidy (i.e. percentage of euploid embryos) was exclusively present in younger women below age 37.5 years ($p=0.001$). Long agonist protocols positively affected percentages of euploid embryos ($p<0.0001$) in comparison to the other two protocols utilized and this effect was statistically visible in younger as well as older women, below and above age 37.5 years ($p<0.01$). **Conclusion:** This preliminary data set suggests that higher gonadotropin dosages result in more euploid embryos for transfer, even though they reduce the percentage of euploid embryos. High dose gonadotropin stimulation, resulting in large embryo numbers, may, therefore, represent a potential indication for PGD. Exogenous LH supplementation, especially in younger women, and long agonist protocols, appear to improve euploidy rates, though a patient selection bias in regards to the selection of stimulation protocols cannot be ruled out. Euploidy rates after ovarian stimulation thus appear quantitatively and qualitatively affected by stimulation protocols and medication dosages."

"Preimplantation Genetic Diagnosis (PGD) Is Indicated for Recurrent Miscarriage Even in Cycles Producing Five or Fewer Embryos." *J. Garrisi, S.H. Chen, N.A. Cekleniak, M.G. Garrisi, J. Cohen, and S. Munne. Institute for Reproductive Medicine and Science at Saint Barnabas, West Orange, New Jersey; Reprogenetics, West Orange, New Jersey.*

According to an abstract presented by the authors at the 62nd Annual Meeting of the American Society for Reproductive Medicine in New Orleans, Louisiana, October 21-25, 2006, "**Objective:** PGD is indicated for patients suffering from infertility or recurrent miscarriage when sufficient numbers of embryos are available. We have studied PGD in groups of patients with low egg yields and varying etiologies. Here we focus on patients with recurrent miscarriages. Previous studies have shown that couples with a history of idiopathic recurrent pregnancy loss (RPL) have a high incidence of chromosomally abnormal embryos. PGD for this indication has proven to significantly reduce the risk of miscarriage while producing high pregnancy rates. However, PGD is a selection tool, and when an IVF cycle produces as many or fewer embryos as the number expected to be replaced, the selection power of PGD is diminished; the same number of normal embryos may be replaced with or without PGD. The objective of this study was to determine whether PGD is indicated in patients producing 5 or fewer embryos. **Design:** This study is a retrospective analysis of PGD patients with recurrent pregnancy loss in an IVF program. **Materials and methods:** Patients with two or more consecutive miscarriages scheduled for PGD were included in the study."

Patients with 5 or fewer embryos that were suitable for biopsy on Day 3 were asked whether or not they wished to continue with PGD analysis. Embryos were determined to be suitable for biopsy if there were at least 4 cells, with fewer than 30% fragmentation. Those who cancelled PGD were considered the 'control' group, and those who decided to proceed were the 'test' group. For the test group, single-cell biopsy was performed on Day 3, followed by fixation and PGD using FISH with probes for 9 chromosomes. Embryo replacement occurred on Day 4 for the test group. Replacement was performed on Day 3 for patients in the control group. Implantation and pregnancy rates were determined by fetal cardiac activity/number of embryos transferred or the number of retrievals. **Results:** A total of 70 RPL couples produced 5 or fewer embryos that were suitable for biopsy. Of those, 47 decided to continue with the PGD procedure. The implantation and delivery rates were significantly higher in the group that received PGD ($p < 0.05$; see Table 1). Although the spontaneous abortion rate was lower in the PGD group (4/22, 18.1% vs. 3/6, 50%), this difference was not statistically significant.

Table 1. Outcomes after PGD of 1-5 embryos for recurrent pregnancy loss patients

	Patients	Average Age	Implantation Rate	Preg/retrieval (%)	Loss Rate (%)	Delivery/retrieval (%)
PGD	47	38.6	20/55 (36.4)	22/47 (46.8)	4/22 (18.1%)	18/47 (36.2)
Control	23	37.5	3/53 (5.6) $p < 0.001$	5/23 (26.1) $p < 0.05$	3/6 (50) $p = \text{NS}$	3/23 (13.6) $p < 0.05$

Conclusion: Even when 5 or fewer embryos were produced, the utilization of PGD was effective in increasing the delivery rate. PGD is therefore indicated for patients with idiopathic RPL irrespective of the number of embryos produced in the cycle. This finding may not be applicable to other poor responder patient groups; the effect of embryo number on other types of poor responders is under active investigation."

"The Role of Geographical Information Systems (GIS) In Fertility Control, Sexual and Reproductive Healthcare." *1B. A. Gbolade and 2R. Maheswaran. 1St James's University Hospital, Fertility Control Unit, Leeds, West Yorkshire, United Kingdom; 2University of Sheffield, Public Health GIS Unit, SchHARR, Sheffield, South Yorkshire, United Kingdom.*

According to an abstract presented by the authors at the 9th Congress of the European Society of Contraception in Istanbul, Turkey, May 3-6, 2006, "**Introduction:** A GIS is a system for capturing, storing, checking, integrating, manipulating, analyzing and displaying data which are spatially referenced to the Earth. Occurrence of significant variations in health over small areas is well known to epidemiologists and health geographers. This phenomenon drives the trend towards provision of primary care services at the local level nationally and internationally, as one step towards providing equal access to health services for those in equal need. However, clinicians are generally not aware of the potential of this technology in their various fields of specialization. **Objective:** To investigate, describe and illustrate the use and importance of GIS in the field of Fertility Control, Sexual and Reproductive Healthcare. **Methods:** A search of electronic medical databases for and review of publications detailing the use and importance of GIS in this field. **Results:** Relatively few numbers of publications were found detailing the use of GIS in these fields. Publications identified include those detailing the use of GIS in assessing access to reproductive health services, investigating HIV heterogeneity and proximity of homestead to roads, examination of the supply of and demand for abortion services, study of the geographic distribution of hospitals with abortion facilities, investigation of travel distance to abortion facilities and geographical variation in abortion ratios, travel distance to abortion facilities and utilization of the facilities by different groups of women, geographical analysis of racial variations in abortion, spatial components of abortion, spatial distribution of teenage conceptions. **Conclusions:** Clearly, there is an emerging role for GIS in fertility control, sexual and reproductive healthcare, especially in health needs assessment, planning and implementation, monitoring and evaluation, resource allocation, surveillance and health impact assessment. Discussion will centre on use of GIS in these areas with adequate illustrations."

Fertility Weekly

International Calendar . . . May 21, 2007

Conferences, Symposia, Meetings Worldwide. *New Listing

- * **August 30-31, 2007**, MADRID, SPAIN, "**International Workshop on Embryo Biopsy and Blastomere Fixation: Hands on Course.**"
Information: Center for Embryo Medicine, Dr Esther Velilla Garcia, Email: pgd@pgdcem.com, www.pgdcem.com.
- * **October 31, 2007 - November 03, 2007**, ANTALYA, TURKEY, "**Middle East Fertility Society, 14th Annual Meeting, MEFS2007.**"
Information: MEFS 2007 Congress Secretariat, P.O.Box 167220 Achrafieh, Beirut-Lebanon, Tel/Fax: 961-1-610400/612400,
Email: registration@mefs.org, <http://www.mefs.org/registration.htm>.
- June 2-5, 2007**, TORONTO, ONTARIO, CANADA, "**The Endocrine Society's 89th Annual Meeting (ENDO).**" Information: The Endocrine Society, 8401 Connecticut Ave., Suite 900, Chevy Chase, MD 20815-5817, Phone (888) 363-6274, Fax (301) 941-0259, Email: societyservices@endo-society.org.
- June 8-9, 2007**, DRAKE HOTEL, CHICAGO, IL, USA, "**Midwest Reproductive Symposium 2007.**" Information: <http://www.cvent.com>, click on RSVP for Event and enter Event Code: 4YN78BST2M3.
- June 20-22, 2007**, VANCOUVER, BRITISH COLUMBIA, "**Workshop on Reproductive Medicine and the Law.**" Information: www.aals.org/events_calendar.php.
- June 21-26, 2007**, OTTAWA, ONTARIO, CANADA, "**Society of Obstetricians and Gynaecologists of Canada (SOGC) 2007.**" Information: sogc.medical.org/index.html.
- July 1-4, 2007**, LYON, FRANCE, "**23rd Annual Meeting of the ESHRE.**" Information: ESHRE Central Office, Van Akenstraat 41, B-1850 Grimbergen, Belgium, Phone +32 2 269 09 69, Fax +32 2 269 56 00, www.eshre.com.
- July 19-21, 2007**, SAN ANTONIO, TX, USA, "**XVth Ovarian Workshop, Ovarian Differentiation, Development, Function, and Persistence.**" Information: <http://www.biosymposia.org>.
- July 21-25, 2007**, SAN ANTONIO, TEXAS, USA, "**40th Annual Meeting of the Society for the Study of Reproduction.**" Information: Gwen Abramson, SSR, 1619 Monroe St., Madison WI, 53711, USA, Tel: 608-256-2777, Fax: 608-256-4610, E-mail: ssr-am@ssr.org, Web: <http://www.ssr.org>.
- August 30 - September 2, 2007**, BERLIN, GERMANY, "**5th European Congress of Reproductive Immunology.**" Information: Aurélie Page, Conventus Congressmanagement & Marketing GmbH, Markt 8, D-07743 Jena, Phone 03641 - 35 33 25, Fax 03641 - 35 33 271, E-mail: aurelie.page@conventus.de, www.conventus.de.
- September 3-7, 2007**, JENA, GERMANY, "**3rd Embryo Implantation Control Summer School.**" Information: Dörte Böttcher, Project Assistant to Udo R. Markert, Friedrich-Schiller-University Jena, Conventus Congressmanagement & Marketing GmbH, Markt 8, 07743 Jena, Germany, Email: doerte.boettcher@conventus.de.
- September 6-8, 2007**, SALVADOR DE BAHIA, BRAZIL, "**Quality Management in Assisted Reproduction.**" Information: Email: info@seronosymposia.org.
- September 8-12, 2007**, HOBART, TASMANIA, AUSTRALIA, "**Fertility Society of Australia - Annual Conference 2007.**" Information: Kim O'Dea, Phone: 03 9645 6311, Email: kimo@wsm.com.au.
- September 9, 2007**, HOBART, TASMANIA, AUSTRALIA, "**Progress of Fertility Preservation in Malignant Disease.**" Information: Email: oceania@seronosymposia.org.
- September 15-19, 2007**, MONTREAL, CANADA, "**14th World Congress on In Vitro Fertilization and 3rd World Congress on In Vitro Maturation.**" Information: ISIVF Congress Secretariat, 687 Pine Avenue West, Rm. F4.23, Montreal, Quebec, H3A 1A1 Canada, Phone: (514) 843-1729, Fax: (514) 843-1678, Email: info@isivf.com, www.isivf.com.
- September 19-21, 2007**, BARCELONA, SPAIN, "**2nd International Congress IVI.**" Information: Noemi de Villasante, Joan Guell, 144, 08028, Tel: +34.93.363.39.51 Fax: +34.93.439.35.94, E-mail: ivicongress@tecnicviajes.com Web: <http://www.congresoivi.com/barcelona/>.
- October 13-17, 2007**, WASHINGTON, D.C., USA, "**63rd Annual Meeting of the American Society for Reproductive Medicine.**"
Information: American Society for Reproductive Medicine, 1209 Montgomery Highway, Birmingham, Alabama 35216-2809, Phone (205) 978-5000, Fax (205) 978-5018, E-mail: asrm@asrm.org, www.asrm.com.
- October 29-31, 2007**, MADRID, SPAIN, "**IVF Preceptorship.**" Information: Email: info@seronosymposia.org.