22 **FETAL DOWN SYNDROME IS ASSOCIATED WITH INCREASED CELL-FREE FETAL DNA LEVELS IN ARCHIVED MATERNAL SERUM SAMPLES** THOMAS LEE[1], ERIK LESHANE[2], GERALYN MESSERLIAN[3], JACOB CANICK[3], MARSHALL CARPENTER[1], WALTER HEBER[1], DIANA BIANCHI[2]; [1]Brown University, Ob/Gyn, Providence, RI; [2]Tufts University, Pediatrics, Boston, MA; [3]Brown University, Pathology and Laboratory Medicine, Providence, RI

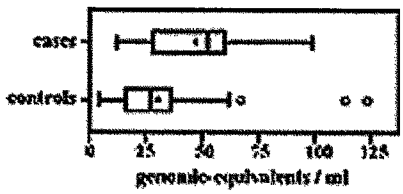
OBJECTIVE: Increased cell-free fetal DNA levels in maternal circulation are a potential noninvasive marker for fetal Down syndrome. While several reports have shown an association between increased fetal DNA levels and trisomy 21 using fresh maternal plasma, a single study using serum has not. The objective of this study was to ascertain if fetal DNA levels are increased in pregnant women carrying fetuses with trisomy 21 using archived serum samples, using a larger number of paired control samples compared with prior studies.

STUDY DESIGN: Routine midtrimester maternal serum screening samples were collected and stored at -20°C from 6 subjects carrying a singleton fetus with 47,XY,+21 karyotype. Each was matched and compared to 5 control samples (30 total) from mothers carrying presumed euploid male fetuses. Controls were matched for gestational age and date of collection. Fetal DNA concentration in maternal serum was quantified using real-time PCR amplification for a Y chromosomal sequence. A mixed model, repeated measures ANOVA was used for statistical analysis.

RESULTS: The adjusted mean fetal DNA concentrations among women carrying trisomy 21 fetuses were 49.7 (95% CI 34.2-65.2) genomic-equivalents/ml (GE/ml) and 30.4 (23.4-37.4) GE/ml among the controls (P = .027). Median values were 50.5 GE/ml and 26.0 GE/ml, respectively.

CONCLUSION: These data suggest that pregnancies complicated by fetal trisomy 21 exhibit 1½-2 fold higher levels of free fetal DNA in midtrimester maternal serum samples and demonstrate that archived serum samples can be a useful source of clinical material for retrospective analyses. Further investigation is necessary before cell-free DNA can be considered as a screening marker for Down syndrome.

Figure
DNA concentration



23 **SELECTIVE TERMINATION (ST) OF ANOMALOUS FETUSES IN MULTIFETAL PREGNANCIES: 200 CASES AT A SINGLE CENTER** KEITH EDDLEMAN[1], JOANNE STONE[1], LAUREN LYNCH[2], RICHARD BERKOWITZ[1]; [1]Mount Sinai School of Medicine, Obstetrics, Gynecology & Reproductive Science, New York, NY; [2]University of Puerto Rico School of Medicine, Obstetrics and Gynecology, San Juan, Puerto Rico

OBJECTIVE: To summarize the outcome of 200 ST procedures performed at a single center.

STUDY DESIGN: 200 patients underwent ST at Mount Sinai Medical Center from 1986-2000 by intracardiac injection of KCl. Under an IRB-approved protocol for data collection, the following data was recorded for each patient: indication for ST, gestational age (GA) at the time of the procedure, starting # of fetuses, ending # of fetuses, which fetus underwent ST (presenting vs. non-presenting), GA at delivery or GA at spontaneous loss < 24 weeks. Outcome data was collected on all 200 patients.

RESULTS: ST was performed on 164 sets of twins, 32 triplets and 4 quadruplets. Average GA at time of ST was 19 wks 2 days (range 12 wks 0 days-23 wks 6 days). 100 (50%) were performed on fetuses with chromosomal abnormalities, 87 (43.5%) with structural anomalies, 7 (3.5%) with Mendelian disorders, 5(2.5%) with severe IUGR +/- oligohydramnios and 1 (0.5%) due to a maternal incompetent cervix. The presenting fetus was terminated in 91 (45.5%) of cases. There were 8 (4%) unintended pregnancy losses <24 weeks, 4/164 (2.4%) in twins, 4/32 (12.5%) in triplets and 0/4 in quadruplets. Time of pregnancy loss averaged 3.0 weeks post-procedure (range 3 days-5 weeks). In 4 (50%) of the unintended losses, the presenting fetus underwent ST and in 4 the non-presenting fetus. Two patients electively terminated their pregnancies after the ST. Average GA at delivery in the remaining patients was 36 weeks 1 day. 160 (84.2%) patients delivered at or beyond 32 weeks gestation.

CONCLUSION: ST at our institution has an overall unintended pregnancy loss rate of 4%. The loss rate is almost 5-fold higher in triplets than in twins. ST is a reasonable alternative in multifetal pregnancies where one fetus has a significant abnormality. This is the largest single center experience in the world.

24 **SKEWED X-CHROMOSOME INACTIVATION AND RECURRENT PREGNANCY LOSS** AMY SULLIVAN[1], TRACEY LEWIS[1], KEN WARD[1], ROBERT SILVER[1], T. FLINT PORTER[1], D. WARE BRANCH[1], [1]University of Utah, Obstetrics and Gynecology, Salt Lake City, UT

OBJECTIVE: In no more than 50% of couples with recurrent spontaneous abortion (RSAB) will a well accepted cause for pregnancy loss be identified. It has recently been suggested that skewed X-chromosome inactivation (SXCI) may be a "molecular phenotype" that identifies patients who carry X-linked recessive disorders. The X-linked disorders are embryo lethal resulting is RSAB. We have tested the hypothesis the SXCI is associated with RSAB in our population.

STUDY DESIGN: We use a case control study design to compare SXCI in women with RSAB. Cases had two or more consecutive pregnancy losses and negative evaluations for RSAB. Controls had at least 2 successful pregnancies and no more than 1 loss. DNA was analyzed for SXCI based on the fact that methylated X chromosomes are inactive, and unmethlyated X chromosomes are active. DNA was digested with Hpa II, an enzyme that digests only unmethlylated (active) DNA. The products were amplified by PCR using flourescent labeled primers and run on an ABI3700. The peak sizes, heights, and areas were analyzed using GeneScan software.

RESULTS: There were 47 women in the RSAB group and 117 controls. The results were informative for 43/47 (91.5%) of cases and 102/117 (87.2%) of controls. Greater than 75% skewing was seen in 10/43 (23.3%) of cases and 27/102 (26.5%) of controls (NS). Extreme skewing (>90%) was not identified in any cases, but was seen in 4/102 (3.9%) of controls.

CONCLUSION: In contrast to other recent studies, we did not find an association between extremely SXCI and RSAB. The percentage of both cases and controls with > 75% SXCI is similar to that found by others. In a highly select group of patients who are negative for traditional causes of RSAB, the molecular phenotype of SXCI may be important.

25 **ENGRAFTMENT POTENTIAL OF HUMAN FETAL HEMATOPOIETIC CELLS IN NOD/SCID MICE IS NOT RESTRICTED TO MITOTICALLY QUIESCENT CELLS** JANNINE WILPSHAAR[1], MICKIE BHATIA[2], HUMPHREY H.H. KANHAI[3], J.H. FREDERIK FALKENBURG[4], EDWARD SROUR[5]; [1]Leiden University Medical Center, Obstetrics/Hematology, Groningen; [2]Robarts Research Institute, London, Ontario, Canada, Hematology, London, Ontario; [3]Leiden University Medical Center, Obstetrics, Leiden; [4]Leiden University Medical Center, Hematology, Leiden; [5]Indiana University School of Medicine, Division of Hematology/Oncology, Indianapolis, IN

OBJECTIVE: During fetal development, there is a continued demand for large numbers of primitive and mature hematopoietic cells. This demand may require that all potential hematopoietic stem cells (HSC) contribute to blood cell production regardless of their position in cell cycle. We recently established that umbilical cord blood cells in G1 phase of cell cycle have a similar repopulating potential as cells in G0, suggesting that cycling prenatal/neonatal HSC may possess the same functional capabilities described for quiescent, but not cycling cells from adult sources.

STUDY DESIGN: To establish the relationship between cell cycle status and hematopoietic potential at early stages of human ontogeny, the in vivo engraftment potential of mitotically defined fetal liver (FL) and fetal bone marrow (FBM) cells were examined in NOD/SCID recipients.

RESULTS: Following transplantation of G0, G1 or S/G2+M CD34+ cells from FL, similar percentages of recipient mice were chimeric (55%, 60% and 60%, respectively). Similarly, FBM-derived CD34+ cells in all phases of cell cycle engrafted in conditioned recipients and sustained human hematopoiesis, albeit at lower levels than similar FL-derived cells. Multilineage differentiation was evident in all transplanted mice independent of the source or cell cycle status of graft cells. In addition, levels of chimerism in mice transplanted with fetal blood-derived G0 or G1 CD34+ lineage-depleted cells were similar.

CONCLUSION: These results support the assertion that both mitotically quiescent, as well as cycling fetal hematopoietic cells, are enriched for putative stem cells capable of multilineage and sustained engraftment in NOD/SCID mouse recipients.