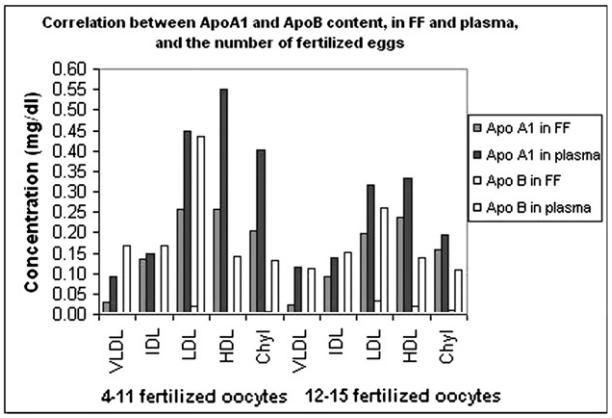
DESIGN: Observational cohort study.

MATERIALS AND METHODS: Plasma and FF from lead follicles ($\geq$16 mm) were obtained from 10 IVF patients at oocyte retrieval. Young ($<$35 years) and older ($\geq$40 years) patients were included. Samples were separated by isopycnic ultracentrifugation into fractions containing HDL, LDL, IDL, and chylomicron (chyl) particles. Quantification of Apo A1 and Apo B was done by ELISA. Cholesterol ester (Chol) and triglyceride (Tg) content was determined by enzymatic colorimetry.

RESULTS: FF Apo A1 is equally distributed in LDL, HDL, and chyl fractions. FF Apo B concentration is significantly lower than Apo A1 and is mostly found in the LDL fraction (Table 1). Plasma Apo A1 and B concentrations and FF Apo A1 are higher in patients with $\leq$11 fertilized oocytes. However, FF Apo B is higher in patients with $\geq$12 fertilized oocytes (Figure 1). FF Chol is present mainly in HDL and chyl fractions and is 4 to 5 times lower than plasma levels. Chol concentration is correlated with the number of fertilized oocytes. Tg are present and are significantly lower in the FF fractions than in plasma.



Figure

TABLE

	Apo A1 (mg/dl)			Apo B (mg/dl)			Cholesterol (mg/dl)		
Fraction	LDL	HDL	Pellet	LDL	HDL	Pellet	LDL	HDL	Pellet
Young n = 5	0.2124 ± 0.1032	0.2300 ± 0.1357	0.2087 ± 0.1228	0.0228 ± 0.0333	0.0128 ± 0.0225	0.0066 ± 0.0116	2.484 ± 1.975	10.214 ± 1.896	5.210 ± 2.023
Old n = 4	0.2683 ± 0.0364	0.2752 ± 0.1122	0.2073 ± 0.1087	0.0261 ± 0.0286	0.0063 ± 0.0029	0.0075 ± 0.0115	2.761 ± 2.719	6.487 ± 4.835	4.049 ± 2.376

CONCLUSIONS: Apo A1 is the primary lipoprotein component in FF. Lower Apo A1 and higher Apo B levels were found in patients with a greater number of mature oocytes. Chol content is likely an indicator of the cellular activities and metabolism in the individual follicular environment, reflecting follicular viability and competence. Tg may not be essential follicular lipid constituents.

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CAN WE PREDICT BLASTOCYST VIABILITY BASED ON DAY 3 EMBRYO DEVELOPMENT? R. Fields, T. Turner, H. Werland, K. Pogson, T. C. Vaughn, K. M. Silverberg. Texas Fertility Center, Austin, TX; Austin IVF, Austin, TX.

OBJECTIVE: Many programs routinely offer Day 3 ET, although Day 5 ET may afford a higher pregnancy rate. As such, it is critical to identify objective selection criteria for Day 3 embryos that offer the best prognosis. Current state of the art dictates embryo selection based on cell number and morphology. However, these characteristics may not best indicate an embryo's ultimate potential. This study was designed to determine how often experienced embryologists can predict which Day 3 embryos become the best blastocysts.

DESIGN: Prospective analysis of all patients undergoing Day 5 ET from 5/2006–3/2007 in a large, private infertility practice.

MATERIALS AND METHODS: Oocytes were inseminated in Sage fertilization medium with 0.5% HSA and cultured in Sage cleavage medium with 10% SPS. Day 3 embryos were evaluated based on cell number, symmetry, and fragmentation. The two best embryos were designated "Day 3 Choice". On Day 5 the two highest quality blastocysts were selected for ET, and supernumerary good quality blastocysts were frozen on Day 5 or 6. Blastocyst selection was based on ICM, trophectoderm, and blastocoel development. Day 3 Choice embryos were compared to the blastocysts ultimately transferred or cryopreserved.

RESULTS: 179 blastocysts from 90 consecutive patients were transferred. Pregnancy and delivery rates were 82.2% and 67%. When evaluating only transferred blastocysts, we successfully predicted both blastocysts 8% of the time; one of two 47% of the time, and neither of the two were identified 45% of the time. When we evaluated all blastocysts that were either transferred or cryopreserved, both of the Day 3 Choice embryos achieved viability 36% of the time, one of the two achieved viability 54% of the time, and neither of the two achieved viability10% of the time.

CONCLUSIONS: Using Day 3 morphologic characteristics, we successfully predicted at least one of the blastocysts that were transferred 55% of the time. This means that almost half of the time, neither of the transferred blastocysts had the best morphologic appearance on Day 3. These data support the primary indication for Day 5 ET - enhanced embryo selection. 90% of the time, at least one of the two best Day 3 embryos were ultimately transferred or cryopreserved. Many of the morphologically best Day 3 embryos, while not selected for Day 5 ET, ultimately become viable blastocysts. Day 3 ET is therefore, a legitimate option, as experienced embryologists can effectively predict ultimate blastocyst viability using these Day 3 criteria.

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EVALUATION OF FERTILITY POTENTIAL BY TOLUIDINE BLUE TEST AND THE SPERM CHROMATIN STRUCTURE ASSAY. R. Mahfouz, A. Agarwal, T. M. Said, J. Erenpreiss, A. Giwercman, R. Sharma. Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics & Gynecology, Cleveland Clinic Foundation, Cleveland, OH; Andrology Lab, Toronto Institute for Reproductive Medicine, Toronto, ON, Canada; Andrology Laboratory, Riga Stradins University, Riga, Latvia; Department of Biology and Microbiology, South Dakota State University, Brookings, SD.

OBJECTIVE: Sperm chromatin integrity is important for mammalian fertilization. The sperm chromatin structure assay (SCSA) using flow cytometry is frequently used for the assessment of fragmented sperm DNA. On the other hand, Toluidine blue (TB) assay is a simple alternate test for sperm chromatin assessment. The objective of our study was to evaluate the Toluidine blue test and SCSA for the assessment of sperm chromatin damage in proven and unproven donors.

DESIGN: Prospective-controlled study.

MATERIALS AND METHODS: Semen samples were collected from 10 unproven donors and 8 proven fertile donors who had initiated a successful pregnancy in the last 2 years. Following liquefaction, seminal ejaculates were evaluated for chromatin abnormalities using Toluidine blue test. In this assay, spermatozoa with abnormal chromatin conformation/DNA integrity) stain dark violet and those with normal chromatin stain light blue. An aliquot was also examined by the SCSA assay for percentage of spermatozoa with immature nuclear development (high DNA stainability index, %HDS).

RESULTS: Proven fertile males showed lower but non significant incidence of spermatozoa with abnormal DNA compared to unproven fertile men by Toluidine blue method (mean $\pm$ SE; 20.9 $\pm$ 4.4 vs. 27.2 $\pm$ 4.9; P=0.09). Similarly, the proven fertile men showed higher incidence of spermatozoa with normal DNA compared to the samples from unproven donor (65.9 $\pm$ 5.2 vs. 52.7 $\pm$ 6.3; P=0.4). In unproven fertile men, %HDS showed a significant positive correlation with the number of spermatozoa staining dark violet (r = 0.85, P=0.002). %HDS was also negatively correlated with the number of light blue sperm (r = $-$0.79, P=0.002).

CONCLUSIONS: Assessment of sperm immaturity is important in the evaluation of fertility potential. While SCSA and Toluidine blue are equally effective tests for sperm immaturity measurement in fertile men, Toluidine blue has an added advantage in identifying nuclear immaturity as well as abnormal chromatin in unproven donors.

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