

gel electrophoresis and only running the non informative allelic combinations in the ABI 310.

Results: Expanded alleles were detected in 100% of the lymphocytes analysed and one PGD cycle was carried out for a semi informative couple. Four embryos were biopsied and only two yielded a PCR product from each of their 2 biopsied blastomeres. In all 4 cells, only the shared allele was detectable after traditional gel electrophoresis. After fluorescent analysis there was no trace of TP amplification in any of the cells and both embryos were transferred. Positive hCG were measured in the first dosage but had decreased in the second. Ten blastomeres were desegregated from the two remaining embryos and all positive cells showed TP-amplified product indicating the presence of the expanded allele in both these embryos.

Conclusions: Reliable detection of expanded CAG repeats was achieved from single lymphocytes and blastomeres using fluorescent TP-PCR. One chemical pregnancy was achieved using this diagnosis technique.

Supported by: This work was supported in part by the association Française contre les myopathies (AFM) and l'assistance publique des hopitaux de Paris (AP-HP).

ART: MALE FACTOR

P-386

Distribution of sperm quantitative/qualitative characteristics from normozoospermic and teratozoospermic specimens in a multi-layer swim-up column. J. R. Correa, P. M. Zavos, P. N. Zarmakoupis-Zavos, R. Fernandez-Pelegrina. Ctr de Fertilidad del Caribe, Rio Piedras, Puerto Rico; Andrology Institute of America, Lexington, KY; Kentucky Ctr for Reproductive Medicine, Lexington, KY.

Objective: Sperm processing for various assisted reproductive technology (ART) procedures is performed to select the best quality spermatozoa and remove factors, such as dead-immotile spermatozoa, debris, WBC's, and others that may interfere with fertilization outcome. Selection of the highest quality sperm population is more critical as the ART method to be performed becomes more complex and the need for using the "elite" spermatozoa is a priority to improve the chances of fertilization and subsequent embryonic development. The sperm processing methods available today separate a semen sample into two populations, one of higher quality than the other. However, sperm subpopulations of higher quality within an already processed sample cannot be separated by those available methods. Recently, a modified swim-up column (ZSC-MC) has been designed with this objective in mind to obtain distinct subpopulations of spermatozoa from a processed semen specimen. Thus, the objective of this study was to assess the recovery and quantitative/qualitative distribution of selected sperm subpopulations from normozoospermic and teratozoospermic specimens.

Design: Selection of sperm subpopulations from normozoospermic and teratozoospermic semen specimens based on sperm swimming ability using a multi-layer swim-up column.

Materials/Methods: Sperm specimens were obtained from 30 normozoospermic and 30 teratozoospermic (but of otherwise normal quantitative/qualitative characteristics) patients. The cutoff point for teratozoospermic status was <30% normal forms by WHO standards. A semen aliquot was placed in the bottom cavity of the ZSC-MC (ZDL, Inc., Lexington, KY), and each of the four conical chambers. Chamber #1 (Ch-1) was the chamber closest to the bottom cavity of the column containing the sperm sample, Chambers #2 and 3 (Ch-2 and Ch-3) were the middle chambers, and chamber #4 (Ch-4) was the top cavity. The cavities were filled with Ham's F-10 medium and incubated at 37°C for 90 min. Semen specimens were assessed for count, motility, forward progression and normal morphology.

Results: Sperm harvesting in the various chambers of the swim-up column resulted in significant improvements in all qualitative characteristics ($P < 0.05$) for both normozoospermic and teratozoospermic specimens. The spermatozoa with the highest qualities improved in each successive chamber from the bottom to the top of the column. The greatest improvements in percentage of normal spermatozoa were realized in teratozoospermic specimens ($P < 0.05$). As expected, there was a significant decrease ($P < 0.05$) in the sperm count harvested in each chamber of the column for both types of specimens due to inherent factors in the selection process.

Conclusions: Sperm harvesting using the multi-layer swim-up column yielded spermatozoa of superior quality than unprocessed specimens for both normozoospermic and teratozoospermic specimens. Furthermore, those improvements in quality increased as the higher quality sperm mi-

grated into the upper cavities of the swim-up column. A sperm subpopulation that best suits the ART procedure of choice may be selected from the appropriate chamber of the multi-chamber swim-up column, which may save time spent in selection of spermatozoa, such as in ICSI cases, and subsequently improve the fertilization outcome and embryonic development.

Supported by: Centro de Fertilidad del Caribe, Inc.

P-387

Testicular sperm retrieval by percutaneous aspiration biopsy using intravenous catheter in azoospermic patients undergoing ICSI. I. M. Fahmy, A. Kamal, M. Aboulghar, R. Mansour, G. Serour. The Egyptian IVF-ET Ctr, Cairo, Egypt.

Objective: Needle aspiration biopsy can be used to retrieve testicular spermatozoa from azoospermic patients undergoing ICSI. In this study we describe a new modification using a wide pore intravenous catheter and compare the results obtained with classical fine needle aspiration (FNA).

Design: Prospective comparative study.

Materials/Methods: The study included 130 azoospermic patients undergoing ICSI (73 with obstructive azoospermia (OA), and 47 with non-obstructive azoospermia (NOA) in whom previous diagnostic biopsy showed foci of normal spermatogenesis or hypospermatogenesis. Patients with pure Sertoli cell only, complete spermatogenic arrest or total hyalinization, were not included. Under local anesthesia, 31 patients with OA and 17 patients with NOA underwent FNA using butterfly needles (G 21-23) and 20 mL syringe to create a negative pressure. For the other patients (42 with OA and 30 with NOA), an intravenous catheter (G 16 or 18) was used instead of the butterfly needle. When testicular tissue was seen coming out, the catheter was clamped and removed gently from the testis. The aspirated fluid or testicular tissue was processed and examined under the inverted microscope. If no spermatozoa suitable for injection were retrieved after a maximum of three punctures, an open biopsy was done.

Results: In patients with OA, successful sperm retrieval was achieved in 41 out of 42 patients (97.6%) using an intravenous catheter compared to 16 out of 31 patients (51.6%) using FNA ($P < 0.05$). In patients with NOA successful sperm retrieval was achieved in 14 out of 30 patients (46.7%) using an intravenous catheter compared to 4 out of 17 patients (23.5%) using FNA ($P < 0.05$). Apart from a hemocele that required surgical evacuation after aspiration by catheter, no other complications were reported.

Conclusions: The use of a wide pore intravenous catheter for testicular aspiration biopsy significantly improved the sperm retrieval rate in patients with OA and NOA.

Supported by: Egyptian IVF-ET Center.

P-388

Differences in seminal parameters between the first and second seminal collection: Can the semen collection method make a difference? P. M. Zavos, J. R. Correa, K. Kaskar, P. N. Zarmakoupis-Zavos. The Kentucky Ctr for Reproductive Medicine and IVF and the Andrology Institute of America, Lexington, KY; Ctr de Fertilidad del Caribe, Rio Piedras, Puerto Rico.

Objective: The collected seminal specimen should, as closely as possible, resemble the ejaculate delivered during intercourse, if the male infertility factor is to be properly identified and treated. Also, the seminal specimen parameters can vary among various collection times within the same individual. The objective of this study was to assess and compare the seminal parameters of two ejaculates collected sequentially either via masturbation (MAST) or at intercourse (MFP) using a non-spermicidal polyurethane condom (Male Factor PakTM).

Design: A prospective, randomized study comparing semen collected via two different methods.

Materials/Methods: A total of 99 patients undergoing male infertility evaluation were used in this study. Patients were randomly allocated into two groups (MAST; $n = 56$; MFP; $n = 43$) and were instructed to provide two specimens each with exactly 4 days of abstinence. Specimens were assessed for volume (mL), total sperm count ($\times 10^6$), percentage and grade of motility, morphology (% normal) and total functional sperm fraction