

MALE REPRODUCTION & UROLOGY

Wednesday, October 25, 2000
2:00 P.M.

O-229

Detection in Mice and Men of a Novel Class of Leukocyte/Macrophages Essential for Normal Development of Reproductive Tract Tissues. A. A. Kiessling, T. E. Mullen, R. L. Kiessling, S. J. Eyre, R. C. Eyre. Laboratory of Reproductive Biology, Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA.

Objective: To identify and characterize cells expressing EMR1-TM7 leukocyte restricted antigens, macrophage members of which have been shown to be necessary for normal development of reproductive tract organs in mice (1).

Design: Male reproductive tract tissues (testis, epididymis, seminal vesicles and prostate) from mice and men were examined for cells expressing surface antigens belonging to the EMR1-TM7 family.

Methods and Materials: Testis, epididymis and seminal vesicles were recovered intact from male mice. Human seminal vesicle and prostatic tissues were recovered at surgery for prostatic carcinoma, and human epididymis recovered from epididymectomy for chronic pain. All tissues were fixed in paraformaldehyde-lysine-periodate, paraffin embedded, sectioned and immunostained with mouse monoclonal antibodies (mAbs) detected by biotinylated second antibody complexed with streptavidin-bound horse radish peroxidase visualized by AEC substrate. The mouse member of the EMR1-TM7 family, F4/80, was detected by a single mAb. Two mAbs were used to detect CD97 (the human homolog to F4/80) in the human tissues, MEM180 and Vim3b. MABs against CD45 were used as a positive control.

Results: F4/80 positive cells were abundant in the interstitium of mouse testis and absent within the seminiferous tubules; CD45 positive cells were rare. Mouse seminal vesicles exhibited both F4/80 and CD45 positive cells specifically on the adluminal side of the epithelium, not within the lumen. F4/80 immunostaining in the mouse epididymis varied markedly according to region; the caput specifically displayed abundant F4/80 positive processes which extended from the basal layer to the lumen, a pattern not observed with CD45 mAb. In contrast, the corpus, cauda and ductus deferens displayed both F4/80 and CD45 positive cells primarily in the basal cell layer and in the interstitium between the ducts. The human tissues exhibited little staining with Vim3b mAb; MEM180 identified numerous cells within the epithelium and the lumen of the human seminal vesicles, but only a few cells in the interstitium in the epididymis and in the prostate. In contrast, CD45 exhibited abundant staining in the human epididymis, with a pattern analogous to the mouse F4/80 in the caput; the CD45 staining in the corpus and cauda was similar to the F4/80 staining in the mouse counterparts. CD45 positive cells were abundant in the seminal vesicles and scattered throughout the prostate.

Conclusions: Tissue specific macrophages, recently identified as essential to normal development of male mouse tissues (Pollard, 1999) are also present in human tissues. EMR1-TM7, a member of the serotonin receptor superfamily, is characterized by an extracellular domain replete with epidermal growth factor receptors which are thought to play an important role in cell-cell interaction. This novel class of macrophages may not only be essential for normal tissue function, but could also serve as host cells for macrophage-tropic human pathogens.

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(1) J. Leuk. Biol., 1999, 66:765-778.

Wednesday, October 25, 2000
2:15 P.M.

O-230

Establishing Normal Oxidative Stress Levels in Population of Proven and Unproven Fertile Men. H. Kobayashi, E. G. Guzman, A. M. Maharan, A. J. Nangia, R. K. Sharma, A. Agarwal. Center for Advanced Research in Human Reproduction and Infertility, The Cleveland Clinic Foundation, Cleveland, OH.

Objectives: Elevated levels of reactive oxygen species (ROS) play an important role in the etiology of male infertility. The imbalance between ROS production and total antioxidant capacity (TAC) in seminal fluid indicates oxidative stress and is correlated with male infertility. Recently,

we found that a composite ROS-TAC score may be more strongly correlated with infertility than ROS or TAC alone. The purpose of this study was to establish normal values of oxidative stress in donors of proven-fertility; normal healthy men of unproven fertility and compare these with a group of patients with male factor infertility.

Design: Prospective study measuring oxidative stress in donors and infertile patients.

Materials and Methods: We analyzed semen samples from 7 donors of proven fertility, 17 healthy donors of unproven fertility, and 61 infertile men diagnosed with varicocele. Semen specimens were processed for ROS measurement by chemiluminescence method. TAC was measured in the seminal plasma by enhanced chemiluminescence assay and a composite ROS-TAC was calculated by principal component analysis.

Results: There were no significant differences between levels of ROS, TAC and ROS-TAC score between donors of proven fertility and donors with unproven fertility.

Variables	Donors of proven fertility (n=7)	Donors of unproven fertility (n=17)	Infertile varicoceles (n=61)	P ^a	P ^b
Log ROS (X10 ⁴ cpm)	1.03 ± 0.56	1.08 ± 0.45	2.13 ± 1.17	0.008	0.0003
TAC (Molar trolox)	1831.25 ± 530.34	1576.88 ± 658.29	1174.29 ± 495.63	0.003	0.007
ROS-TAC score	52.90 ± 9.94	48.81 ± 10.07	26.47 ± 19.92	0.0003	0.0001

Values are expressed as mean ± SD; ^a P<0.05 significant by pairwise comparison of proven donors with infertile varicocele patients; ^b P<0.05 significant by pairwise comparison of donors of unproven fertility with infertile varicocele patients.

Conclusions: Seminal oxidative stress is minimal in population of normal men with proven or unproven fertility. Our study establishes the levels of seminal oxidative stress in a population of normal donors. Others can use these results as a reference value to compare their population of normal and infertile men.

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Wednesday, October 25, 2000
2:30 P.M.

O-231

Single Large Testicular Biopsy is Comparable to Multiple Biopsies to Retrieve Spermatozoa in Patients with Non-Obstructive Azoospermia. I. Fahmy, A. Kamal, R. T. Mansour, G. I. Serour, M. Aboulghar. The Egyptian IVF-ET Center, Maadi, Cairo, Egypt.

Objectives: Various techniques have been proposed to retrieve spermatozoa for intracytoplasmic sperm injection (ICSI) in patients with non-obstructive azoospermia. While random multiple sampling is routinely performed in many centers, others prefer to obtain one large biopsy assuming that focal spermatogenesis is randomly distributed throughout the testis. The present study aimed to compare the result of testicular sperm extraction (TESE) using both techniques.

Design: Prospective comparative study.

Materials and Methods: In all patients with non-obstructive azoospermia undergoing TESE combined with ICSI, we routinely take an initial small biopsy. If this sample did not show spermatozoa, further biopsies were taken. The study included 89 patients in whom the first sample did not reveal spermatozoa. These patients were randomly divided into two groups. In the first group (n=45), the testicular wound was extended and a large piece of testicular tissue was removed. Another large biopsy was taken from the contralateral testis. In the second group (n=44), further two or three biopsies were obtained from each testis depending on testicular size and the presence of adhesions. About 250-500 mg of tissue was removed in each case. All the samples were weighed and processed routinely. If inadequate numbers spermatozoa suitable for injection were found, late spermatids were used for injection if found.

Results: The mean weight of tissue removed did not differ significantly between the two groups (p>.05). In the single-biopsy group, ICSI was performed using spermatozoa in eight patients, late spermatids in two

patients and both in three patients. In the multiple-biopsies group, ICSI was performed using spermatozoa in eight patients, late spermatids in one patient and both in three patients. The overall rate of sperm and late spermatid retrieval was 13/45 (29.5%) for the single biopsy group and 12/44 (26.7%) for the multiple biopsies group ($p>0.05$). Two patients from the single-biopsy group and three patients from the multiple-biopsies group developed moderate hematoma. No other complications were noted.

Conclusion: In patients with non-obstructive azoospermia undergoing TESE in whom the first testicular sample did not show spermatozoa, a single large bilateral testicular biopsy gave sperm retrieval rates comparable to those obtained by bilateral multiple biopsies provided that an equal amount of testicular tissue is removed.

Wednesday, October 25, 2000
2:45 P.M.

O-232

Simplified and Safe Approach with High Yield for Testicular Sperm Recovery by Fine Needle Aspiration, Followed by Intracytoplasmic Sperm Injection for the Treatment of Non-Obstructive Azoospermia.

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Objectives: To summarize our 5 years experience with testicular fine needle aspiration (TEFNA) for sperm recovery in non-obstructive azoospermia.

Design: A retrospective chart review of all men suffering from non-obstructive azoospermia operated for sperm recovery in our unit from January 1995 to December 1999.

Materials and Methods: A total of 152 men underwent 236 TEFNA operations, consisting of a mean of 15 punctures and aspirations in each testis, using 23 gauge butterfly needles, connected to a 20 ml syringe with an aspiration handle. Following the initial TEFNA, a second intervention was performed in 51 men, a third in 20 men, a fourth in 6 men, a fifth in 4 men and one patient underwent 8 consecutive procedures, leading to a total of 236 TEFNA cycles. Classified by testicular histology, the study population comprised men diagnosed as suffering from Sertoli cells only (SC) in 56 (36.8%) cases, maturation arrest (MA) in 38 (25.0%) cases, hypospermatogenesis (HS) in 36 (23.7%) cases, tubular hyalinization (TH) due to non-mosaic Klinefelter's syndrome (47, XXY) in 20 (13.2%) cases, and post-irradiation fibrosis (IF) in two (1.3%) cases.

Results: Mature testicular spermatozoa could be recovered in 127 (53.8%) cycles. The recovery rate by testicular histology was 42 out of 87 (48.3%) in the patients with SC, 22 out of 57 (38.6%) in MA, 52 out of 63 (82.6%) in HS, 11 out of 27 (40.7%) in TH due to Klinefelter's syndrome, whereas no spermatozoa were found in the two cases with IF. ICSI was performed in all 127 cycles. Transfer of embryos deriving from 2PN fertilization was performed in 84 cycles, resulting in 26 ongoing pregnancies, a pregnancy rate of 31%. No complications or major side effects were recorded.

Conclusion: TEFNA was found to be highly efficient, easy to learn, safe and well tolerated by patients. We considered TEFNA as the first choice approach whenever sperm recovery is attempted in non-obstructive azoospermia.

Wednesday, October 25, 2000
3:00 P.M.

O-233

Testicular Pathology Discovered in Candidates for Testicular Extraction of Sperm. L. Kaufman, C. S. Niederberger, L. S. Ross. Chicago, IL.

Objectives: The development of Intracytoplasmic Sperm Injection (ICSI) made possible direct retrieval of sperm from testes for use in Artificial Reproductive Techniques. At times, the urologist is not involved in the evaluation and/or retrieval of sperm in the infertile male. We found significant testicular pathology in patients who were candidates for testicular sperm extraction. These would have been discovered with an appropriate

urological evaluation. Failure to detect these entities prior to TESE would have compromised patient care and possibly lead to medical legal action.

Methods: 73 patient charts from candidates undergoing TESE during a 1 year time period were reviewed. Patients excluded from TESE due to testicular pathology were recorded.

Results: 2 patients (2.7%) were excluded from TESE due to testicular pathology. The first, a 32-year-old male with a history of left testicular torsion, left orchiectomy, and fixation of the right testis, presented with azoospermia. Serum assay demonstrated FSH 57mIU/ml, LH 20 mIU/ml, and testosterone 22 ng/dl. Physical exam revealed absent left testis, palpable right vas and firm large right testis. Scrotal ultrasound was performed, revealing a right testicular prosthesis. The second patient, a 33-year-old male, was evaluated 1 year earlier, and found to have 100,000 sperm/ml, bilateral testicular atrophy (3.7 cm), serum FSH 35.5 mIU/ml and testosterone 627 ng/dl. One cycle of IVF failed, 1 year elapsed, and the patient presented with continued oligospermia and was scheduled for TESE. A small firm mass was felt in the posterior aspect of his left testicle. Ultrasound revealed an intratesticular mass which was revealed to be seminoma on radical orchiectomy.

Conclusion: In 1994, Jarow reported finding potentially life-threatening medical pathology in 1.1% of 1236 patients presenting for evaluation of male infertility. The development and widespread use of ICSI coupled with the ability to use testicular derived sperm has sometimes led to the omission of Urologists from the clinical reproductive algorithm. Our findings of testicular pathology in candidates for testicular sperm extraction support the imperative place for the Urologist in the evaluation and treatment of the infertile male.

Wednesday, October 25, 2000
3:45 P.M.

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Size of Varicocele Corresponds with Pregnancy Rates Following Surgical Treatment: Updated Results. ¹J. I. Sandlow, ¹M. Zenni, ¹J. Ehle,

³J. F. Donovan, Jr., ^{1,2}A. E. T. Sparks. From the Departments of ¹Urology and ²Obstetrics and Gynecology at the University of Iowa, Iowa City, IA, and ³Department of Urology, University of Oklahoma, Oklahoma City, OK.

Objectives: To examine the effect of the size of varicoceles on postoperative results, including change in seminal parameters, strict morphology, and pregnancy rates.

Design: A retrospective chart review and telephone follow-up of all patients undergoing varix ligation at our institution between 1995 and 1999.

Materials and Methods: Retrospective review of 104 patients with clinical varicoceles prior to repair. We examined the preoperative physical findings of varicocele size, as well as semen analysis and strict morphology results. All patients in this study were required to have at least 1 preoperative and 1 postoperative seminal fluid analysis (SFA) at our institution prior to varix ligation. Patients were stratified into unilateral small (U-S), bilateral small (B-S), unilateral moderate or large (U-M/L), and bilateral moderate or large on at least one side (B-M/L). We then correlated this to postoperative outcome, including change in seminal parameters, strict morphology, and pregnancy rate, both natural and assisted.

Results: A total of 104 patients were evaluated. Twenty-three had U-S, 10 had B-S, 30 had U-M/L and 41 had B-M/L varicoceles. When evaluated for improvement in total motile sperm per ejaculate (TME), 10/23 (43%) U-S, 6/10 (60%) B-S, 19/30 (63%) U-M/L, and 22/41 (54%) B-M/L improved, for an overall rate of improvement of 55%. When examining pregnancy data, the overall natural pregnancy rate was 43/104 (41%). However, the rate of natural pregnancy was 2/23 (9%) for U-S, as compared to 4/10 (40%), 20/30 (67%), and 17/41 (41%) for B-S, U-M/L, and B-M/L, respectively ($p<0.0002$). A total of 31 couples underwent in vitro fertilization, with 21 achieving pregnancy (68%). The improvement in the percentage of strictly morphologically normal sperm (overall 38%) did not seem to correlate with the size of the varicocele. Preoperative TME did not predict response to repair or likelihood of conception.

Conclusions: The repair of all clinical varicoceles, except for small unilateral varicoceles, results in a significant improvement in seminal parameters. Natural conception rates were significantly higher for all groups as compared to small unilateral varicoceles. Based upon these data, we recommend varix ligation for all men with clinically moderate or large varicoceles, as well as bilateral small varicoceles.