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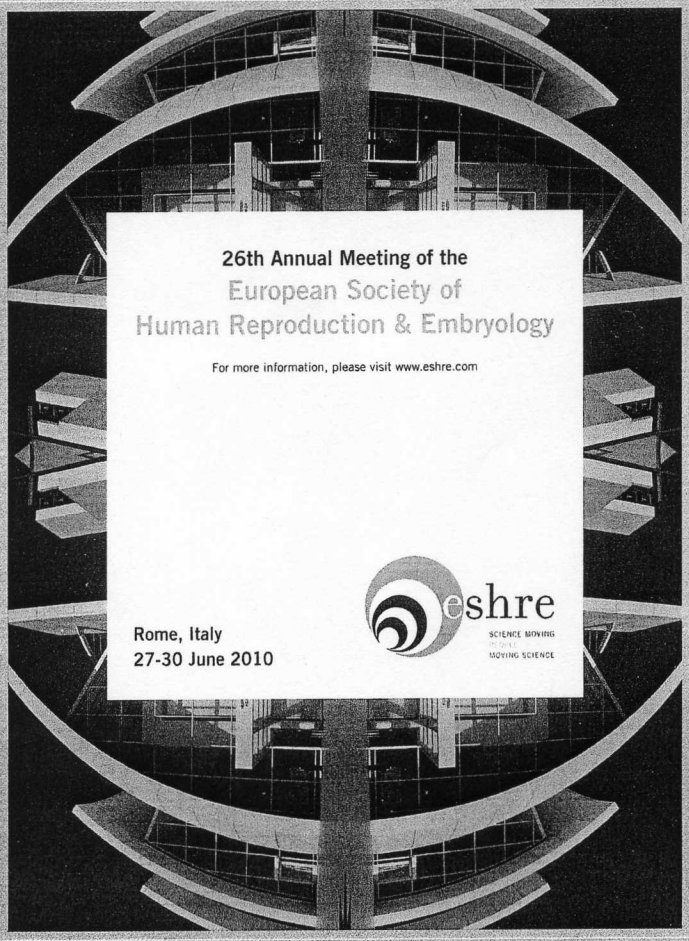

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linked to cellular events respondent to androgens. The hormonal action of the two most important androgens (testosterone and 5-αdihydrotestosterone) is mediated by the action of the androgen receptor (AR). In fact, the AR is a nuclear transcription factor code by the AR genes. The aim of this study was to molecularly characterize the mutations and the CAG polimorphisms in a population of infertile men who lives in a rural region, with the diagnosis of idiopathic infertility.

Material and Methods: In this prospective study, we compared 45 rural men with idiopathic infertility with 45 rural men who had a previous child in the last year. In the group of infertile men, all women had a child from a previous relationship. All semen samples were collected in the same room and evaluated by the same person. Semen specimens were collected after 3–4 days of abstinence. Samples were then analyzed for sperm concentration and percent progressive motility according to World Health Organization (WHO) criteria and sperm morphology according to Tygerberg's strict criteria. DNA was obtained from the linfocytes' blood and from cells of the oral mucosa through smear provided in a commercial kit (Wizard Genomic Purification) used to evaluate the AR. Statistical analysis was performed using the Student t test. Results were described as mean ± SD.

Results: Differences were detected in the semen analysis in the group of infertile men compared to normal semen donors: sperm concentration (mean ± SD) (30.1 ± 36.5 vs. 57.1 ± 23.2; p = 0.03), sperm motility (40.93 ± 20.7 vs. 52.3 ± 14.5; p = 0.03), sperm morphology according to the WHO (10.7 ± 6.55 vs. 36.5 ± 4.2; p = 0.03) and Tygerberg's strict criteria (3.8 ± 2.8 vs. 8.3 ± 3.1 p = 0.03) No differences were found on CAG in the group of infertile patients when compared to the normal healthy men (20.04 ± 3.93 vs. 20.64 ± 3.71, respectively; p = 0.09). In the group of infertile men, 12 alleles were identified; the most common were the 20 (17.8%) and the 21 (20%) CAG, both identified in 8 patients. In the control group, 13 alleles were found; the 18 (15.55%) and the 21 (20%) were the most common, being identified in 7 and 9 patients, respectively. No correlation was found between the polymorphism CAG and sperm concentration (r = 0.227; p = 0.134), sperm motility (r = 0.202; p = 0.184), and sperm morphology according to the Tygerberg's strict criteria (r = 0.210; p = 0.213). A weak association between the CAG repetitions and sperm morphology according to the WHO was found (r = 0.349; p = 0.032). The mean of CAG repetitions in the group of patients with severe oligospermia was higher (22.2 ± 3.59) than the control group (20.64 ± 3.71; p = 0.03). No differences were detected in the number of CAG repetitions in the group of normal fertile men or infertile men with normospermia compared to the group of infertile men with oligospermia, teratospermia or asthenospermia (p = 0.09).

Conclusions: Our results suggest that, in agriculture patients with severe oligospermia, the evaluation of the AR may be an important tool in order to detect the reason of the infertility. Studies with more patients must be performed to confirm our data.

P-030 Varicocelectomy impact on assited reproductive approach

E. Borges Jr.[1], F.F. Pasqualotto[2], R.C.S. Figueira[3], A.S. Setti[4], D.P.A.F. Braga[4], S.S. Cortezzi[4], A. Iaconelli Jr.[1]

[1]*Fertility – Assisted Fertilization Center, Clinical Department, Sao Paulo, Brazil*

[2]*Caxias do Sul University, Biotechnology Department, Sao Paulo, Brazil*

[3]*Fertility - Assisted Fertilization Center, IVF Laboratory, Sao Paulo, Brazil*

[4]*Fertility - Assisted Fertilization Center, Scientific Research, Sao Paulo, Brazil*

Introduction: Male factor is responsible itself for 20% of the total infertility cases, contributing to other 30-40% of the cases. Varicocele plays an important role in this scenario, affecting 40% of infertile men. It is also present in approximately 10-20% of normal male population. Varicocelectomy has been considered the infertility treatment of choice since 1952, when the first spontaneous post-varicocelectomy pregnancy was reported. *The Practice Committee of the American Society for Reproductive Medicine* indicates surgical repair of varicocele when an adult has a palpable varicocele and is unable to achieve pregnancy in subsequent attempts, or has an abnormal semen analyses or desires future fertility. However, even when varicocele is repaired and all beneficial effects over semen quality appears to be obtained, unassisted pregnancy rates vary from 19% to 35%. Therefore, assisted reproductive technologies (ART) are important for the management of couples with male factor infertility associated with varicocele, even in cases of men who underwent varicocelectomy. The aim of this study was to evaluate the contribution of varicocelectomy, proposed before infertility treatment, in improving the sperm quality and consequently the outcomes of ART.

Material and Methods: Data from 248 patients submitted to intracytoplasmic sperm injection (ICSI) cycle, performed from 2000 to 2008, were retrospectively evaluated and subdivided in two groups: men with varicocele who did not perform surgical repair (Group 1, N = 79) and those who did performed varicocelectomy (Group 2, N = 169) before ICSI. All cases of female infertility were excluded. Ejaculated samples were assessed regarding semen parameters according to WHO, and Tygerberg's strict criteria was used to carry out sperm morphology analysis. We also evaluated maternal and paternal age, period of infertility, number of previous ICSI attempts, testicular volume, oocyte yield, fertilization rate, number of embryos transferred, implantation, pregnancy and miscarriage rates in both groups.

Results: No significant differences were found between the two groups regarding demography and cycle's general characteristics. Similar maternal age (33.0 ± 4.6 *vs* 33.8 ± 3.8, P = 0.1872), total dose of recombinant follicle stimulating hormone (FSH) administered (2371 ± 785 *vs* 2249 ± 640, P = 0.2943), estradiol (E2) levels on the day of hCG administration (1926 ± 364 *vs* 2002 ± 201, P = 0.8490), number of aspirated follicles (17.3 ± 12.5 *vs* 16.4 ± 10.5, P = 0.9752), oocyte yield (75.4% *vs* 77.6%; P = 0.6401), and metaphase II (MII) oocyte rate (61.2% *vs* 66.4%; P = 0.6217) were observed. However, our results indicate that paternal age (36.1 ± 5.5 *vs* 37.8 ± 4.7, P = 0.0319) and period of infertility (2.7 ± 0.3 *vs* 6.0 ± 4.9, P < 0.001) were significantly higher in men who underwent varicocelectomy. Although semen volume was higher in Group 1 (3.3 ± 0.3 *vs* 2.5 ± 0.14, P = 0.0043), sperm concentration (30.08 ± 4.01 *vs* 24.1 ± 2.42; P = 0.138), progressive motility (38.2 ± 2.69 *vs* 38.7 ± 2.08; P = 0.881), as well as normal morphology (2.6 ± 0.44 *vs* 2.4 ± 0.37; P = 0.7202) were similar between groups. Fertilization rate was significantly higher in Group 1 (73.2% *vs* 64.9%, P = 0.0377). However, no differences were observed on pregnancy (31.1% *vs* 30.9%; p = 0.9806), implantation (22.1% *vs* 17.3%; P = 0.5882) and miscarriage rates (21.7% *vs* 23.9%; P = 0.8401) in Groups 1 and 2.

Conclusion: Although varicocelectomy has become the most commonly performed operation in regards to male infertility, its benefits on the restoration of testicular function, as well as fertility potential are controversial. Even though a varicocelectomy should always be performed before indicating assisted reproduction in order to achieve a pregnancy with sexual intercourse, our results suggest that this surgical procedure does not have any significant impact on ICSI outcomes. In addition, our results showed that when submitted to an ART, men who underwent varicocelectomy are older, with higher period of infertility than those not submitted to varicocele repair. Therefore, ART could be considered an important alternative in the management of couples with clinical varicocele and suboptimal seminal parameters, without benefit of previous surgical treatment.

P-031 Seminal vesicle ultrasonographic characterization in infertile patients with type 2 diabetes mellitus

S. La Vignera[1], E. Vicari[1], M. Di Mauro[1], N. Burrello[1], R. Condorelli[1], R. D'Agata[1], A.E. Calogero[1]

[1]*University of Catania, Department of Biomedical Sciences, Catania, Italy*

Introduction: Ejaculatory dysfunction is common in type 2 diabetes mellitus (DM) and it is a potential cause of infertility in these patients. The lack of studies exploring the ultrasonographic characteristics of the seminal vesicles (SVs) in diabetic patients and the high frequency of neuropathy in these patients, which also affects SV function, prompted us to evaluate the ultrasound characteristics of the SVs in infertile patients with DM. To accomplish this, prostate-vesicular transrectal ultrasonography (TRUS) was performed in infertile patients with type 2 DM without any known cause of infertility. Patients with idiopathic infertility and infertility plus male accessory gland infection (MAGI) were selected a control and a well-defined clinical model of SV inflammation.

Methods: A total of 75 infertile patients were selected for this study according to the following criteria: 1) Control group: 25 infertile patients without DM and no known causes of infertility. They had a mean ± SEM age of 35 ± 0.5 years (range 28-37, years); 2) MAGI group: 25 infertile patients without DM but with MAGI, diagnosed according to the WHO criteria (1993). They had a mean ± SEM age of 31 ± 0.6 years (range 27-36, years); 3) DM group: 25 infertile patients with type 2 DM and no other known causes of sperm parameter abnormalities. They had a mean ± SEM age of 34 ± 0.8 years (range 28-36,

years). All patients underwent TRUS examination after 1 day of sexual abstinence, before and after 1 hour from ejaculation. The prostate-vesicular region was assessed using a transrectal 7.5 MHz biplan biconvex transducer (Esaote GPX Megas, Genova, Italy) and the following SV ultrasound parameters were recorded from each patient, by the same operator: 1) body antero-posterior diameter (ADP); 2) fundus APD; 3) parietal thickness of the right and left SVs; 4) number of polycyclic areas within both SVs; 5) fundus/body ratio; 6) difference of the parietal thickness between the right and the left SV; and 7) pre- and post-ejaculatory APD difference.

Results: Patients with MAGI and DM had a significantly higher SV body and fundus APD which was similar to each other. Patients with DM had a significantly higher F/B ratio compared to controls and patients with MAGI. Parietal thickness was significantly higher only in patients with MAGI. On the other hand, the difference in the parietal was significantly higher in both patients with MAGI and DM compared to controls, but patients with DM had significantly lower difference compared to patients with MAGI. Only patients with MAGI had a significantly higher number of polycyclic areas. Finally, controls and MAGI patients has a similar pre- and post-ejaculatory difference of the body SV APD, whereas this difference was significantly lower in patients with DM.

Discussion: The SV of infertile DM patients showed ultrasound features that differentiate them from the other two groups of infertile patients. First of all, DM patient SVs did not have the characteristic claviform conformation, as shown by the increased fundus/body ratio (>2.5) observed in about 70% of them. A second peculiar aspect was the asymmetry of the parietal thickness between the two SVs which suggests the presence of an inflammatory component in the SV of DM patients, although to a lower degree compared to patients with MAGI. The presence of a lower inflammatory component was further highlighted by a normal number of polycyclic areas (honeycomb aspect) in DM patients. Finally, they had an unchanged post-ejaculatory APD. In conclusion, this study showed that infertile patients with DM have SV which show morphological abnormalities suggestive of functional atony likely as a result of the diabetic neuropathy. This may be an additional factor playing a pathogenetic role in the fertility disturbances experienced by the patients with DM.

P-032 Identification and functional characterization of a point mutation in the gene encoding the sperm factor phospholipase C zeta in an infertile male

J. Kashir[1], C. Jones[1], C. Young[2], M. Ruas[2], P. Grasa[2], K. Rietdorf[2], E. Heytens[3], B. Heindryckx[3], S.Y. Yoon[4], R.A. Fissore[4], C.M. Deane[5], D. Nikiforaki[1], S.T. Tee[1], P. de Sutter[3], J. Parrington[2], K. Coward[1]

[1]*University of Oxford, Nuffield Department of Obstetrics and Gynaecology, Oxford, United Kingdom*

[2]*University of Oxford, Department of Pharmacology, Oxford, United Kingdom*

[3]*Ghent University Hospital, Department of Reproductive Medicine, Ghent, Belgium*

[4]*University of Massachusetts, Department of Veterinary and Animal Sciences, Amherst, U.S.A.*

[5]*University of Oxford, Department of Statistics, Oxford, United Kingdom*

Introduction: Mammalian oocytes are thought to be activated by a sperm-specific phospholipase C, PLCzeta (PLCζ). Whilst some types of human male infertility appear to be caused by failure of the sperm to activate the oocyte, it remains unclear as to whether this is due to defective PLCζ. Here, we describe the identification of a point mutation in the catalytic region of the PLCζ gene in one infertile male diagnosed with oocyte activation deficiency. The consequences of this mutation upon functional ability were investigated using mammalian cell expression and mouse oocyte injection.

Materials and Methods: PLCζ exons were amplified by polymerase chain reaction from fertile/infertile males, sequenced, and compared with PLCζ wild-type (WT) and cDNA sequences. Constructs were engineered in which WT and mutant (M) PLCζ were fused with a fluorescent mCherry tag. An additional construct was created in which the C-terminal region of PLCζ (partial Y and entire C2 domain) had been intentionally deleted (ΔC2). In order to assess oocyte activation and calcium (Ca^{2+}) release ability, WT- and M-PLCζ cRNAs were microinjected into mouse oocytes. We also assessed HEK293T cells transfected with WT-, M-, and ΔC2-PLCζ for their ability to respond to ATP, a potent agonist of Ca^{2+} release. The expression and localization of the three PLCζ isoforms were studied in HEK293T cells using fluorescent and confocal microscopy. DAPI and Wheat Germ Agglutinin-GFP conjugate were used as markers of the nucleus and plasma membrane, respectively.

Results: DNA analysis revealed a point mutation (His >Pro) within the catalytic domain of PLCζ in one infertile male with normal sperm morphology. Computer modelling indicated significant effect upon protein structure and ability to interact with other proteins. Whilst all mouse oocytes injected with WT-PLCζ cRNA exhibited Ca^{2+} oscillations characteristic of fertilization, those injected with M-PLCζ cRNA failed to respond. Live cell imaging of HEK293T cells demonstrated that the Ca^{2+} response for cells expressing WT-PLCζ was significantly diminished compared to that of M- and ΔC2-PLCζ, resembling the Ca^{2+} response observed in cells transfected with constructs absent of PLCζ. Mean amplitudes were significantly different at 5μM (WT: 1.6, M: 2.8, ΔC2: 3.1), and 100μM ATP (WT: 1.9, M: 3.6, ΔC2: 3.2). The percentage of responding cells was significantly lower at 5μM (WT: 26%, M: 83%, ΔC2: 88%) and 100μM ATP (WT: 21%, M: 86%, ΔC2: 93%). Fluorescent and confocal imaging of transfected HEK293T cells demonstrated that the three constructs exhibited cytoplasmic localization with clear differences in expression levels (WT >M > ΔC2-PLCζ).

Conclusions: Our study identified, for the first time, a single PLCζ point mutation (His >Pro) in an infertile male diagnosed with oocyte activation deficiency. Computer modeling suggested significantly impaired 3D structure and ability to interact with other proteins. Concomitantly, M-PLCζ cRNA was unable to cause Ca^{2+} release, or induce activation upon injection into mouse oocytes. HEK293T cells transfected with M-PLCζ revealed reduced levels of expression when compared to those transfected with WT-PLCζ. Cells transfected with the ΔC2-PLCζ construct expressed levels of mCherry fluorescence which were significantly lower than the WT- or M-PLCζ constructs. Further experiments demonstrated that HEK293T cells expressing WT-PLCζ failed to release Ca^{2+} when stimulated with ATP, indicating that over-expression of active WT-PLCζ may have exhausted internal Ca^{2+} stores, unlike the M- or ΔC2-PLCζ constructs. Our studies indicate that defective forms of PLCζ may play an important role in certain types of male factor infertility and suggests that an active recombinant PLCζ may represent a valuable therapeutic tool for such patients.

P-033 A comprehensive mutation screen of genes involved in sperm motility

L. Visser[1], G.H. Westerveld[1], S.K.M. van Daalen[1], F. van der Veen[1], M.P. Lombardi[2], S. Repping[1]

[1]*Academic Medical Center, Obstetrics & Gynaecology, Amsterdam ZO, The Netherlands*

[2]*Academic Medical Center, Clinical Genetics, Amsterdam ZO, The Netherlands*

Introduction: Adequate motility is crucial for the fertilizing potential of sperm. Impaired motility of human sperm cells, i.e. asthenozoospermia, is detected in ~20% of couples presenting with infertility. Although genetic causes are thought to underlie asthenozoospermia, none have been identified so far. Mouse knockout models and in vitro functional experiments have put forward a number of candidate genes for asthenozoospermia in humans.

Material and Methods: We performed a case-control study and screened 30 men suffering from isolated asthenozoospermia and 90 controls for mutations in the coding regions of nine asthenozoospermia candidate genes: *AKAP4, CATSPER1, CATSPER2, CATSPER3, CATSPER4, GAPDHS, PLA2G6, ADCY10* and *SLC9A10.* Whenever mutations were found, their putative impact was evaluated *in silico.* To account for a possible effect of heterozygous mutations, we assessed imprinting of all candidate genes by studying the expression pattern of heterozygous SNPs in testis biopsies of five unrelated men.

Results: We identified 10 asthenozoospermia-specific mutations in *AKAP4* (n = 1), *CATSPER1-4* (n = 6), *ADYC10* (n = 2) and *PLA2G6* (n = 1). These mutations were distributed over six patients, with one patient carrying three mutations, two patients carrying two mutations each and three patients having a single mutation. None of these mutations were found in controls. *In silico* analysis showed that eight of the mutations had a negative BLOSUM score, were located in conserved residues and/or were located in a functional domain. All mutations were heterozygous and expression analysis demonstrated that *CATSPER 1* and *4* are imprinted.

Conclusions: Given their putative effect on protein structure, their location in conserved sequences and/or functional domains, and their absence in controls, the identified mutations in *AKAP4, CATSPER2, CATSPER3, CATSPER4,*