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OC 05

A SINGLE NUCLEOTIDE POLYMORPHISM (SNP) OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) GENE (GLU298ASP VARIANT) IN INFERTILE MEN WITH ASTHENOZOOSPERMIA

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Objective(s): Nitrogen monoxide (•NO), is a highly reactive free radical gas generated in bio-logical systems. Lewis et al. reported that NO is synthesized by the human male gamete. Excessive NO concentrations can cause defective sperm function, low and controlled concentrations of NO play an important role in the control of sperm physiology. Nitric oxide synthase endothelial enzyme (eNOS) produce NO by oxidation of L-arginine (L-Arg). Our aim was to examine the possible association(s) between the eNOS missense mutation (Glu298Asp variant) in infertile patients with asthenozoospermia and sperm motion kinetics. Methods: investigated the frequency of the G894T polymorphism (Glu298Asp variant) of the eNOS gene in 70 infertile men and in 60 healthy men. Sperm motion kinetics were assessed with computer assisted semen analysis (CASA). Presence of T>G, a single nucleotide polymorphism (SNP) in exon 7 of the eNOS gene was determined by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis. Sequencing analysis was used to confirm the specific genotype. Finding(s): The G894T eNOS (T) allele was found at a higher frequency in the patients with asthenozoospermia (60% vs. 22.5% in the control group) (P = 0.02). The percentage of progressive motile sperm (grade a+b) was lower in the asthenozoospermic infertile men with the homozygote eNOS (TT) genotyping than in the wild-type eNOS (GG) (p = 0.02) and in the heterozygote eNOS (GT) genotyping (p = 0.01). However, the percentage of progressive motile sperm (grade a+b) was higher in the wild-type vs the mutant eNOS (TT) (p=0.03) and heterozygous eNOS (GT) genotyping (p=0.04). Conclusion(s): Our findings suggest that the T allele encoding for aspartic acid of the eNOS (Glu298Asp) gene may contribute to poor sperm motility.

OC 06

APOLIPOPROTEIN E POLYMORPHISMS IN MALE FERTILITY

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Apolipoproteins (Apo) are components of lipoproteins which are molec aggregates that have a lipidic transport function and help redistribute choles and triglycerides to the cells and different organs. They consist in five sep classes, ApoA, B,C, D and E. Apo E is produced by several human cells, suc hepatocytes, macrophages, spermatocytes and smooth muscle cells of the te This wide distribution suggests additional functions besides the transpor cholesterol, such as the hormonal regulation of homeostasis in various organs. Apo E exhibits a genetic polymorphism with three codominant alleles: e*2, and e*4, whose products, the isoforms E2, E3, and E4 differ for two amino located in position 112 and 158 (Arg/Cys). The aim of our study was to evaluat three isoforms of Apo E in an infertile population to correlate the qualit spermatogenesis with different polymorphisms. We employed the RFLP. We the enzyme HhaI, which identifies the mutations in position 112 and 15 selected 338 patients divided into three groups: azoospermic (oligozoospermic (128) and normozoospermic (105).

Genotypic and allelic frequencies are reported in Table I (a, b).

Tab I a	Azoo	OAT	Normo
ApoE Genotypes			
E*3/E*3	72,3%	69,5%	69,5%
E*3/E*4	18 %	19,5 %	19%
E*2/E*3	6,6 %	7%	9,5%
E*2/E*4	1,9%	3,9%	2,8%
E*4/E*4	0,9 %	1,5%	0

Tab I b	Azoo	OAT	Norm
ApoE Alleles			
ApoE*2	4,3%	4,7 %	5,7
ApoE*3	84,8%	82,8 %	83,4
ApoE*4	10,9%	12,5 %	8,5

The analysis using X ² test of allelic and genic frequencies did not det significant change in genotypic and allelic frequencies in the three groups stu compared to the frequencies of the Italian population. In literature there ar populationistic papers that studied the relationship between ApoE polymorpl and fertility. These studies have assessed the fertility on the basis of the numl children born and found a positive relationship between E * 2 allele anc reproductive capacity, and between E * 3 and high fertility. However, there a studies on the distribution of ApoE in different seminal phenotypes. Our data that ApoE polymorphisms are not associated with semen quality; the impa these polymorphisms is similar in cases of altered spermtogenesis, su azoospermia, that in normozoospermic subjects, showing once again that the "number of children born" as an index of fertility is incorrect.

OC 07

EVALUATION OF CONVENTIONAL AND BIOFUNCTIONAL SPERM PARAMETERS IN INFERTILE PATIENTS WITH HEPATITIS B AND C VIRUS INFECTION

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Introduction. Hepatitis B (HBV) and C (HCV) infection are a serious global public health problem. Many studies have shown that HBV passes the blood-testis barrier, penetrates male germ cells and integrates into their genome, while HCV is involved in many extrahepatic alterations, very likely due to an increased chronic inflammatory response. However, the mechanisms by which these two viruses impact on human spermatozoa remain unclear. Aim of this study was to evaluate sperm conventional and biofunctional parameters in infertile patients with HBV or HCV infection. **Methods.** Ten infertile patients (20-45 years, mean 32) in Child-Pugh A classification with HBV, and 10 patients (22-42 years, mean 31) with HCV, underwent sperm analysis and flow-cytometric evaluation after quantitative detection of HBV-DNA or HCV-RNA in blood serum. The following biofunctional sperm parameters were evaluated by flow-cytometry: DNA fragmentation by TUNEL, mitochondrial membrane potential (MMP) following JC1 staining, chromatin condensation following sperm permeabilization and propidium iodide (PI) staining, phosphatidylserine (PS) externalization after annexin V and PI staining. Results were compared with those of 20 fertile men (20-45 years, mean 34). **Results.** Patients with hepatitis had sperm conventional and biofunctional parameters significantly worse than controls (sperm density: 31.7 vs. 80.4 mil/ml; forward motility: 9.4 vs. 25%; normal forms: 15.4 vs. 24.8%; DNA fragmentation: 10.5 vs. 2.2%; low MMP: 50 vs. 8%; PS externalization: 5 vs. 2.7%; abnormal chromatin: 18.9 vs. 13.9%). HBV patients had sperm conventional parameters significantly worse than those found in patients with HCV, except morphology (sperm density: 39.5 vs. 23.8 mil/ml; forward motility: 5 vs. 13.7%; normal forms: 16.5 vs. 14.3%). Patients with HBV or HCV infection had a similar percentage of spermatozoa with low MMP, fragmented DNA and PS externalization, whereas HCV patients had a significantly higher percentage of spermatozoa with abnormal chromatin than HBV patients (21 vs. 15.7%). **Discussion.** These results showed that HBV or HCV infection has a negative impact on sperm conventional parameters, mitochondrial function and chromatin/DNA integrity. To our knowledge, this is the first study assessing the effects of HBV or HCV on biofunctional sperm parameters.

OC 08

CXCR7 RECEPTOR EXPRESSION INCREASES DURING PROST CANCER PROGRESSION

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Background: The chemokine CXCL12/stromal cell-derived factor-1 (SDF-1) receptors CXCR4 and CXCR7 play a major role in tumor invasion, proliferatio metastasis. Aim: (1) To document if CXCR7 receptor is differentially expressed prostate cancer (Pca) progression; (2) To identify the therapeutic potential of a C receptor antagonist (CCX733) on preclinical models of androgen independer Methods: The CXCR7 expression was evaluated in: (i) human Pca tissues from radical prostatectomy (50 cases); (ii) human Pca bone metastases (6 case Cell clones with increased osteotropic potential; (iv) Pca tissues as well as l nodes metastases generated after ortotopical injection of DU145 and PC3 cel The therapeuthical potential of CCX733 was invetigated in several Pca cell lin migration, invasion and anoikis were used as outcome measures of efficacy. In human Pca tissues CXCR7 expression was higher in undifferentiated Pca (G 8-10) with a further increase in bone lesions. The association between C expression and osteotropic potential was further documented in vitro models. In cells with increased osteotropism (C4-2b, PC3b1 and PC3b2) the CXCR7 exp was higher than parental cell lines. Accordingly with these evidences, high le CXCR7 was documented in locoregional lynphnodes derived from orthotopic model. Finally xenografts of 22rv1 injected subcutaneosly with human oste expressed CXCR7 with an evident positivity in tumor cells, endothelial ce osteoblasts (both resting and proliferating). The theraputic potential of a CCX7 investigated on vitro models. Our results indicate that CCX733 (i) decreases bot and SDF1/OBCM induced cell migration and invasion and this is partially medi the reduction of MMP-9 and uPA secretion and activity; (ii) reduces both ba SDF1 induced cell growth with an IC50 ranged from 50 to 250 nM and (iii) in the anoikis (apoptosis induced in absence of adhesion) known to be one of th events of metastatic process. Conclusions: Taken together our results indicate a CXCR7 in Pca progression and suggest a potential role of CXCR7 antagonism management of advanced androgen independent Pca.