## 1061

### REDUCED SPERM QUALITY AND – QUANTITY IS ASSOCIATED WITH REDUCED TESTICULAR BLOOD FLOW AND HIGH LEVELS OF DIET DEPENDENT SERUM HOMOCYSTEINE

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**INTRODUCTION & OBJECTIVES:** Homocysteine is considered as a risk factor for atherosclerosis, in particular for cardiovascular disease and placental vasculopathy. Besides suggested genetic factors, plasma homocysteine levels are strongly influenced by diet. Furthermore, testicular blood flow is essential for sperm quality and quantity, as well as a high resistive index (RI) in testicular blood flow was found to be associated with pathological sperm count. Our objective was to measure homocysteine levels in plasma and testicular blood flow from infertile men with pathological sperm count for unknown reasons. Furthermore, we evaluated sperm quality and quantity before and after therapy with a homocysteine lowering diet.

**MATERIAL & METHODS:** A total of 40 patients (mean age 34.3 years, range 21-44) with without known risk factors for infertility and pathological sperm count were included in this pilot study. All subjects underwent complete andrological screening. Semen samples were analysed according to the WHO (1999) guidelines. Measurement of the testicle RI (normal range 0.45 – 0.59) was performed using a high-frequency Doppler ultrasound probe (10 MHz, Acuson Sequoia 512). Three RI measurements were performed on each testicle at an intratesticular artery. Serum homocysteine measurement (normal range 6.3 – 11.2 µmol/l) and semen analysis were performed before and after a three month treatment with a vitamin combination (*Folic acid:* 300µg, *Pyridoxin*(Vit. $B_6$): 3.0 mg, *Cyanocobalamin* (Vit. $B_{12}$): 3.0µg) once daily.

**RESULTS:** An elevated pre-therapeutical mean serum homocysteine level (13.5 µmol/l) was found in this group of patients, as well as a reduced testicular blood flow expressed by an elevated mean RI (0.66). After three months therapy, patients demonstrated an significantly (p=0.006) increased sperm count from 43.9 Mil./ml (pre-therapy) to 55.1 Mil./ml (post-therapy) and a significant (p=0.0001) increase in motility from 18.9% (pre-therapy) to 27.6% (post-therapy) of progressive sperm. The mean serum homocysteine levels decreased to 9.66µmol/l (p=0.0001).
In 18 patients with a pre-therapeutical sperm count of less than 20 Mil/ml a significant (p=0.004) increase in sperm quantity (pre therapy mean 9.4 Mil./ml, post therapy mean 23.8 Mil./ml) and a significant (p=0.0001) increase in quality (pre therapy mean 18.9% progressive sperm, post therapy 31.5% progressive sperm) was observed.

**CONCLUSIONS:** In accordance to hitherto studies serum homocysteine levels seem to be a possible marker for reduced blood flow in the testicle as well as in other organs. Furthermore, supplementation of homocysteine lowering vitamins seems to lead to an increase of sperm quality and quantity, even in patients with severe pathological sperm count. Further studies in larger groups of patients are urgently needed to investigate the relationship between homocysteine, testicular blood flow, sperm quality/quantity and diet.

## 1062

### SIGNIFICANCE OF PRE-IMPLANTATION GENETIC DIAGNOSIS IN AZOOSPERMIC PATIENTS

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**INTRODUCTION & OBJECTIVES:** Injection of sperm has been used to treat male infertility caused by obstructive and non-obstructive azoospermia. Some data show significantly lower pregnancy rate in couples where testicular spermatozoa from non-obstructive azoospermic men were used. This could be explained by a higher aneuploidy rate in embryos. The group can benefit from Preimplantation Genetic Diagnosis for Aneuploidy Screening (PGD - AS) by blastomere analysis.

**MATERIAL & METHODS:** We conducted, therefore, a prospective study in period 05/2003 – 10/2004 offering all couples with non-obstructive azoospermic (NOA) partner ICSI in combination with PGD-AS. Our aim was to compare the aneuploidy rate of embryos and reproductive outcome in TESE group versus other PGD – AS groups (age, implantation failure, ...). In patient with a clinical diagnosis of NOA microsurgical TESE were performed. Larger seminiferous tubules (probably with active spermatogenesis) were selectively excised, microdissected and examined for presence of spermatozoa. Obtained sperms were frozen; the samples were thawed and used for ICSI in dependence of female partners stimulation. Embryos reaching at least 5-cell stage on day 3 were biopsied. The individually biopsied blastomeres were spread onto slide. A two-round FISH procedure allowing to detect chromosomes X, Y, 13, 16, 18, 21 and 22 was performed; at the last 6 cycles was add chromosome 15. A retrospective analysis involved 14 TESE - PGD cycles in patient with normal karyotype.

**RESULTS:** Altogether 109 embryos were analyzed in this group: 46.8% were euploid for detected chromosomes. Embryo transfer was performed in 90% of cases; pregnancy rate per cycle has reached 55%. That is almost twice higher than in other groups included to PGD. In control group (26 TESE cycles without PGD) the pregnancy rate per cycle is 23%.

**CONCLUSIONS:** The results clarify that non-obstructive azoospermic patients profit from PGD-AS and this method should become standard part of ART in this group.

## 1063

### AUTOMATED MEASUREMENT OF TOTAL ANTIOXIDANT CAPACITY OF SEMINAL PLASMA

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**INTRODUCTION & OBJECTIVES:** There is a balance between oxidants and antioxidants in organism. While oxidants increase, antioxidants are consumed. If the antioxidants are not sufficient against the oxidants, the balance shifts to oxidants, consequently oxidative stress occurs, which play a role in the etiopathogenesis of more than 100 disorders. Although there are some measurement methods to evaluate total antioxidant capacity (TAC) of seminal plasma, none method is appropriate for automated measurement in routine laboratory except more recently developed method by Erel. In this study, we measured semen TAC and compared with a most widely used commercial method, Randox -TAS, and oxidative and antioxidative parameters of seminal plasma.

**MATERIAL & METHODS:** TAC values of semen samples were measured by both Randox -TAS assay and the novel assay measuring direct TAC using ABTS radical cation. Individual antioxidant parameters of semen; thiol content, vitamin C, albumin, globulin, uric acid and bilirubin were also measured. Oxidative status of seminal plasma was evaluated by measuring total peroxide, malondialdehyde and lipid hydroperoxide levels. The relationships among the measured parameters were analyzed using correlation test.

**RESULTS:** There was an important correlation between the novel automated TAC measurement method and Randox-TAS assay ($r= 0.92$, $p<0.0001$). Vitamin C showed a significant correlation with TAC ($r= 0.69$, $p<0.0001$). There was a significant correlation between TAC and thiol content ($r= 0.45$, $p<0.0001$). On the other hand, negative correlations between TAC and total peroxide ($r=-0.23$ $p=0.04$), malondialdehyde ($r= -0.28$, $p=0.03$) and lipid hydroperoxide ($r= -0.26$, $p=0.04$) levels were determined.

**CONCLUSIONS:** The more recently developed fully automated TAC measurement method correctly determines TAC of seminal plasma, and it can be readily used to measure TAC of seminal plasma in routine activities or investigations.

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### A NOVEL COLOUR DOPPLER ULTRASOUND ASSISTED NO-SCALPEL TESTIS BIOPSY

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**INTRODUCTION & OBJECTIVES:** Two sharp dissecting clamps that were routinely used in standard no-scalpel vasectomy were used to accomplish this novel testis biopsy technique.

**MATERIAL & METHODS:** 53 Adult infertile males accepted no-scalpel testis biopsy for diagnostic or therapeutic purposes. Among them, 22 underwent left testis biopsy, 12 with right testis biopsy and the remaining19 underwent bilateral testis biopsy. *A brief introduction on the surgical technique:* Two sharp dissecting clampsthat are routinely used in standard no-scalpel vasectomyare required. The procedure should be conducted under cord block and local infiltration anaesthesia using 1% plain lidocaine. The target testis is held firmly with the scrotal skin stretched over its anterior surface. Before the injection of subcutaneous local anaesthesia agent, all acceptors must receive the colour Doppler ultrasound examination to identify the capsular and sub capsular vessel-free area. Then 1% plain lidocaine is injected into the stretched subcutaneous region just above sub capsular vessel-free area of the target testis. Pierce the stretched, puffyscrotal skin with the dissecting clamp and dilate the wound to approach the underlying tunica vaginalis. The underlying tunica vaginalis was pickedup to confirm the location, afterwards, use the otherdissecting clamp to pick up the other point of elevated tunica vaginalis to expose the plane between the two pick-up points. Cut the transverse plane vertically to expose the underlying tunica albuginea and then repeat the above pick-up and cutting procedures to expose the underlying testicular tissue. The seminiferous tubules can be clearly seen and the volume of testicular tissue can be retrieved as demanded. Finally, the 2 tunica layers are sutured interruptedly and keeps the skin wound unclosed.

**RESULTS:** All the 53 acceptors underwent ambulatory testis biopsy by using this technique, the testis tissue retrieval rate was 100% with various diagnosis. There were no postoperative wound infection or hematoma noticed after a 3 month-follow-up. The mean operation time was significantly shortened than the other traditional open biopsy group. The mean time of wound healing was 3.5±0.2 days, which was also significantly shorter, more importantly, the mean wound dimension (8.1±2.1mm) was compatible with the documented standard no-scalpel vasectomy.

**CONCLUSIONS:** With the help of colour Doppler ultrasound, this technique has the advantages in easier, or at least, similar assessment of the testicular tissue as the standard open testis biopsy method, but it retains the advantages of minimal invasion and excellent cosmetics as found in the percutaneous testicular biopsy and the testicular fine needle aspiration.