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Posters

(PO 001 to PO 116)

PO 001

Obesity and male reproduction function

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Objective : Reproductive function may be affected by environmental and occupational exposures as well as changing lifestyle. However, whether or not these factors contribute to an increasing risk of male reproductive health problems including poor semen quality is stirring an ongoing debate. On the other hand, obesity is becoming increasingly prevalent worldwide and is now considered to be one of the most important health concerns in many countries. Notably, the impact of the woman´s body mass index (BMI) on fecundity is well established, whereas only few reports addressed the potential reproductive hazards of obesity in males. Therefore, this study aimed at investigating the relationship between BMI and parameters of reproductive function in men attending an infertility clinic.

Patients and Methods : The retrospective study included a total of 496 men undergoing andrological examination for infertility work-up. Apart from infertility, inclusion criteria comprised age (18-50 years), BMI >18.5 kg/m^2, uneventful andrological history, and normal genital examination. Patients with genetic or congenital abnormalities, genital disorders, systemic disease, therapeutic use or abuse of drugs, as well as seminal signs of inflammation or obstruction were excluded. Semen analysis had been performed according to WHO guidelines extended by biochemical markers and microbiology. For statistical analysis, patients were categorized according to BMI considering cigarette smoking and positive seminal bacteriology as confounders.

Results : A normal BMI (>18.5 to <25 kg/m^2) was found in 209 men (42.1%), 287 patients (57.9%) showed a BMI >25 kg/m^2. Compared to the general population in Germany, the prevalence of obesity was markedly increased, with the highest difference among the subgroups of men aged 25-29 years and 30-34 years (39.7 vs 61.2% and 48.3 vs 62.3%, respectively). Semen analysis revealed normozoospermia in 35.4% of men with normal weight, but only in 25.4% of those with a BMI >25 kg/m2. However, this trend was not significant and also the confounding factors showed no marked effect on semen quality. A significant negative correlation could be observed between BMI and serum testosterone, whereas gonadotrophin levels remained unaffected.

Conclusions : The results confirm previous reports describing a decrease of serum testosterone with increasing BMI. The high prevalence of obesity among men attending an infertility clinic suggests that this factor may contribute to male reproductive health problems, although the trend towards deterioration of semen quality with increasing BMI is not significant. With regard to the alarming prevalence of obesity among adolescents, this issue might become increasingly important in clinical andrology in order to prevent at least some cases of male subfertility.

PO 002

Incidence of dysspermia categories in a contemporary diagnostic setting

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Objective : To classify sperm disturbances in relation to possible aetiological factors using the standard clinical approach, employing all available diagnostic tools and

following WHO's guidelines for categorization (W.H.O., 2000).

Design : Analysis of the clinical material of an Endocrinology Department embedded Andrology Clinic of a busy inner-city hospital in Athens for classification of sperm disturbances was made using the diagnostic categories proposed by W.H.O. but expanded to include new classes not previously reported (e.g. epididymopathies, multifactorial causes, etc.).

Materials-Methods : A total of 774 cases investigated for couple subfertility and found to be dysspermic have been selected from a large database from which normozoospermic men have been excluded. The diagnostic classification was based on meticulous history taking, physical examination, semen analysis (W.H.O., 1999 criteria,) imaging techniques, endocrine tests (including inhibin-B), and when needed, testicular biopsy and chromosome and molecular analysis.

Results : On the basis of the factors identified these cases were groupped into 3 major categories: (a) single-factor group (37.3%), (b) two-factor group (34.0%) and (c) three or more factors group (28.27%).

In the single-factor group, the incidence recorded was in the following order: 1. Idiopathic OTA (40.6%), 2. varicocele (18.7%), 3. epididymopathy (12.8%), 4. environmental (8.0%), 5. infections (5.3%), 6. acquired testicular damage (4.8%), 7. congenital anomalies (3.2%), 8. systemic causes (2.1%), 9. endocrine causes (1.6%), 10. sexual-ejaculatory dysfunction (1.3%) and four other diagnostic groups with less than 1% or no representation. In the two-factors group, epididymopathy (31.3%), varicocele (26.5%) and environmental (20.6%) factors were the most frequently encountered components of the various combinations. Finally, in the three or more factors group, the main components were environmental (24.8%), varicocele (19.2%) and epididymopathy (19.0%).

As it is obvious, in single factor aetiology idiopathic OTA was the principal category whereas in all multifactorial combinations varicocele, environmental factors and epididymopathy were dominating the field.

Conclusions : The diagnostic categories formulated in this analysis differ significantly from the relevant data presented from the much larger series from other centers (Nieschlag, 2001). In our material, multiple factors have been recognized in the great majority of the cases and the single-factor category was clearly in a minority. It appears that the detailed diagnostic work-up with the introduction of new investigational parameters was instrumental in explaining, at least in part, the marked differences between the two series. On the other hand, one should also count the differences between the relevant populations in terms of environment, prevailing health conditions, socioeconomic status, etc. As it becomes obvious, studies charting each population's patterns of dysspermia should be available in each different population as a prerequisite for a sound reproductive policy for the male.

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PO 003

Interests of the post coital test in the investigation of the infertile couples

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Objective : to establish the profile of the cervical mucus and the sperm to infertile couples of the Tunisian centre.

Design and place : Service of Cytogenetic and Biology of Reproduction, University Hospital F Hached Sousse, Tunisia.

Methods : Our study concerns 49 infertile couples which benefited of at least: a postcoital test (PCT) and a spermogram. Test was realized in meadow ovulatory period among the 11-th and the 12-th day of the cycle after an average sexual intercourse of 8 hours and a 3 days average sexual abstinence.

The exo cervical mucus is taken with an a bit long crowbar, we measures the filance between the two branches of the crowbar; on the other hand the endocervical mucus is taken by means of an aspiglaire. The endo and exo cervical mucus are examined under microscope between blade and small strip.

Results : The majority of the couples (63%) benefited from a hormonal treatment before the taking of the cervical mucus. Only 18% of the patients benefited during the PCT of a double taking endo and exocervical. Post coïtal test was positive in 89% of cases and negative in 11% of cases. Only 12% of the patients have cervical infertility and a single patient has an azoospermia.

Conclusion : post-coïtal test is a test of reliable diagnosis to infertile couples because according to our results the majority of the patients who benefited from PCT are porters of cervical infertility.

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PO 004

The place of Doppler ultrasonography in evaluating male infertility

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Introduction and Objectives : Male infertility may be the cause of several disorders, so that identifying a correct diagnosis and an appropriate treatment may represent a challenge for the clinician. Our goal was to evaluate in a retrospective study the correlations between spermatic parameters, scrotal abnormalities and the resistive index in infertile males, as determined by Doppler ultrasonography.

Patients and methods : Our study group consists of 56 infertile males, aged between 21 and 46 years old, and 40 fertile males, as a control group. The evaluation protocol included andrological examination, hormonal profile, and determination of antispermatic antibodies, prostatic and testicular Doppler ultrasonography.

Results : Testicle hypotrophy was diagnosed in 40.7% cases, 26% patients presented cysts or calcifications of the epydidimus, 32% had chronic prostatitis, 12% had spermatocystitis, 10% had testicular tumors. 29.64% of the infertile patients had olygospermia. Varicocele was present in 71.42% of cases, and in 31.57% cases the varicocele was bilateral – stage I varicocele in 26.6%, stage II in 40% and stage III in 33.4%. Doppler ultrasound combined with the Valsalva maneuver identified three grades of reflux : grade I in 22.2% cases, grade II in 35.5% cases and grade III in 42.3% cases. The mean value of the resistive index in the control group was 0.56, in patients with varicocele the mean value was 0.46, and in patients with azoospermia the mean value was 0.90. The severity of the spermatogenetic dysfunction was better correlated with the grade of reflux than with the clinical stage of varicocele.

Conclusions : Varicocele is the cause of infertility in about 50% of the patients studied. The severity of olygospermia is correlated with the grade of the venous reflux. Doppler ultrasonography is an essential diagnostic tool before surgery and a valuable measure of postoperative results.

PO 005

Epidemiologic features of varicocele in a large cohort of Greek men with infertility

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Objective : Varicocele is a common cause of male infertility and one of the most controversial issues in the field of Andrology. The main aim of this study was to analyze the epidemiologic, clinical, hormonal and sperm parameters in a large cohort of infertile men with varicocele in a northern Greek population. A secondary aim was to detect changes of these parameters in men who underwent surgical repair of varicocele.

Design : Retrospective, epidemiologic, descriptive, clinical study.

Materials and Methods : We accessed medical records of 925 infertile men that were examined in our outpatient clinics between 1991 and 2005 ; 429 (46%) of them having either a clinical varicocele or a surgically repaired varicocele were included in the study. Studied parameters included age of male and female partners, type and duration of infertility, testicular volume, side and grade of varicocele, FSH, LH, prolactin, testosterone and sperm parameters before and after the surgical repair where available. Of the 429 men, in 272 (64%) varicocele was the only cause of infertility, whereas other additional causes included infection (n=77 ; 18%), idiopathic non-obstructive azoospermia (INOA) (n=40 ; 9%), cryptorchidism (n=16 ; 4%), obstruction (n=7 ; 2%) and other causes (n=17 ; 3%).

Results : Studied parameters are illustrated in the table. Results are given as mean ± standard deviation or percentage.

In the subgroup of men (n=87) who underwent surgical repair of varicocele, no statistically significant changes were found in sperm and hormonal parameters after the operation. The lack of statistical significance remained when we analyzed separately men with varicocele only and men with varicocele plus INOA.

	All men	Varicocele only	Varicocele and INOA
Age of men (years)	33.5 ± 6.3	33.5 ± 6.2	32.7 ± 6.5
Age of women (years)	33.3 ± 5.6	33.1 ± 6.1	32.2 ± 4.3
Type of infertility			
primary	75%	75%	84%
secondary	25%	25%	16%
Duration of infertility (years)	3.8 ± 3.6	3.5 ± 3.0	4.2 ± 4.1
Right testis volume (mL)	21 ± 5	22 ± 3	15 ± 7
Left testis volume (mL)	20 ± 6	21 ± 4	12 ± 7
Varicocele side			
left	61%	63%	54%
right	4%	2%	22%
bilateral	35%	35%	24%
Varicocele grade			
grade I	11%	10%	9%
grade II	85%	87%	82%
grade III	4%	3%	9%
FSH (mIU/mL)	9.3 ± 9.5	6.9 ± 4.0	16.4 ± 14.7
LH (mIU/mL)	6.8 ± 4.8	6.0 ± 4.0	9.3 ± 5.7
Prolactin (ng/mL)	8.4 ± 6.3	7.3 ± 5.2	10.7 ± 6.6
Testosterone (ng/dL)	505 ± 221	533 ± 228	427 ± 241
Sperm volume (mL)	3.9 ± 1.8	3.9 ± 1.7	3.5 ± 1.5
Sperm number (10^6/mL)	32.3 ± 42	36.5 ± 46.2	7.5 ± 17.0
Sperm motility (%)	30.5 ± 22	33.3 ± 22.4	15.3 ± 19.5
Sperm morphology (%)	28.8 ± 21	30.2 ± 21.2	17.8 ± 17.9

Conclusions : Varicocele is a very common finding in infertile men, although an etiologic relationship between varicocele and male infertility is difficult to be established. According to our findings, surgical repair does not seem to be generally effective, thus it should be applied only in a meticulously selected group of men.

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PO 006

The varicocele - a frequent cause of male infertility

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Introduction : Although the pathophisiology of the varicocele is a well known matter, its correlation with male infertility and with the optimal timing of surgery in men with associated spermatogenesis abnormalities and decreased spermatic motility is still controversial. Our objective is to asses the consequences of the varicocele on the reproductive function.

Material and Methods : 22 infertile men, aged 20 - 45 years old, with varicocele and normal hormone levels, were included. The evaluation protocol included an andrological examination, the evaluation of hormonal status, two spermograms in a three months interval, antispermatic antibody measurement, sperm cultures, cytogenetic exam and testicular pulse Doppler ultrasound. Venous reflux associated with the Valsalva maneuver was also determined. Patients were reassessed 2, 4 and 6 months after surgery for varicocele, by seminal liquid analysis, antispermatic antibodies measurement. Also, ultrasonography was routinely performed.

Results : Antispermatic antibodies were present in 77.8% of the infertile patients, significantly correlated with the grade of the venous reflux and less dependant on the clinical stage of the varicocele. The spermograms showed a wide range of abnormalities, from astenospermia to severe oligo-asthenoteratozoospermia. These abnormalities were more severe in patients with intratesticular varicocele and high grade venous reflux. After surgery, the testicle hypotrophy stopped its progression in all patients. Ultrasound evaluation 2 months after surgery showed the disappearance of the venous reflux at the Valsalva maneuver in 90% of cases. All patients who induced spontaneous pregnancies were under 35 years old.

Conclusions : Varicocele induces autoimmune processes, with an increase in spermatic antibodies. Surgery for varicocele significantly increased the fertility potential in men under 35 years old. Future research is needed in order to reveal the interdependent mechanisms involved in spermatogenesis abnormalities and decreased spermatic motility noted in males with varicocele.

PO 007

Effect of varicocele on the formation of antisperm antibodies "ASA"

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The exact etiopathology through which varicocele can affect fertility potential is still unknown.

This work aims to study the effect of varicocele on ASA formation and its correlation with the stress pattern detected in cases of varicocele.

The study included two groups: thirty evident varicocele (grades II and III) infertile patient (group I) and fifteen varicocele-free fertile, age matched volunteers as a control group (group II).

Both patients and controls were subjected to; standard semen analysis, expressed prostatic secretion examination; excluding those having chronic prostatitis and detection of both serum and seminal plasma ASA by ELISA.

The results showed that; the varicocele patients have highly significant low sperm density and percent of actively motile sperm ($p<0.001$) and significantly higher level of abnormal forms ($p < 0.05$) compared to controls.

The results also showed that; in varicocele group; both serum and seminal plasma ASA are negatively correlated with sperm density ($r=-0.732$ and -0.66 - $p < 0.001$) respectively, and percent of actively motile sperms ($r=-0.739$ and -0.771 - $p < 0.001$) respectively, whereas serum and seminal plasma ASA are positively correlated ($r=0.685$, $p<0.001$), denoting that seminal plasma ASA are derived from the systemic circulation.

In conclusion; varicocele can precipitate to the formation of ASA. The latter can be considered as one of the etiopathogenic mechanisms through which varicocele can affect the fertility potential.

PO 008

Primary varicocele and fecundity : Post surgical assessment of sperm and fecundity parameters

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Objectives : To assess the post operative evolution of male infertility (semen analysis parameters) and the fertility outcomes.

Materials and Methods : We underwent a retrospective study including 50 patients with a varicocele operated according to Palomo procedure in the department of Urology of Hôpital Général de Grand Yoff. The parameters studied were related to semen analysis (density, mobility in the first hour and the vitality) and the spermocytogramme (count of normal spermatozoa). This analysis was done once before the operation and twice after the operation (between the 3rd and 8th month and from the 9th month).

Results : The semen analysis found an improvement of all the parameters for all the patients without normalization except for the morphology. The post operative outcome was statistically significant for the density. The majority of the patients (76% to 92%) had abnormal preoperative values of semen except for the morphology (28%). Semen analysis parameters were improved for 48% to 64% of patients depending on the parameter. For patients with abnormal semen, the lower the mean preoperative value of a parameter, the higher it becomes postoperatively. The post operative pregnancy rate was 31,42%. The fertile patients were those with a younger age, a shorter duration of infertility, constantly improved semen analysis parameters compared to infertile patients.

Conclusion : Values and improvement of semen analysis parameters are more favourable in patients with young age and those with bilateral varicocele and a secondary infertility.

Keywords: Fertility, Semen analysis, Varicocele repair.

PO 009

Varicocelectomy and scrotal temperatures in infertile men with varicocele

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Objectives : Increased scrotal temperature is one of the various factors either associated with or considered as a cause of impaired spermatogenesis in infertile men with varicocele. Some studies have reported a reduced scrotal temperature after varicocelectomy. We investigated whether this finding was true, and if such a reduction in temperature persisted over time after surgery.

Materials and Methods : This retrospective study included 2 groups of infertile patients with a left varicocele: an operated group of 116 patients, and a non-operated group of 40 patients included in an intrauterine insemination programme (IUI). Varicocele was diagnosed by clinical examination (grade I: Valsalva positive ; grade II: palpable; grade III: visible). In both groups clinical examination was performed before surgery

or before inclusion in the IUI programme (T0) and again at 3 months (T3) in both groups, and at 6 months (T6) after surgery in the operated group. Scrotal temperatures were measured on each side with a special thermometer with the patient naked and in a supine position for at least 10 minutes.

Results : Comparison of scrotal temperature values at T0 and T3 revealed no significant change in left and right mean temperatures in the non-operated group, while both left and right mean temperatures decreased significantly in the operated group.

In the operated group, comparison of scrotal temperature values at T0, T3 and T6 indicated that left and right mean temperatures were significantly lower at T3 and T6, with no difference between T3 and T6 values.

Considering a value <35.1°C as the upper limit of normal scrotal temperature, an abnormal temperature (>35.1°C) was observed in 51% (59/116) of the operated group before surgery. In this subgroup of patients with abnormal temperature before surgery, temperature was normal in 86% (51/59) 3 months after surgery. In the subgroup of patients with normal temperature before surgery, scrotal temperature was still normal in 91% (52/57) but abnormal in 9% (5/57).

Conclusions : Surgery induced a reduction in mean scrotal temperature in a group of infertile patients with a left varicocele. This reduction was indeed the result of surgery, as mean scrotal temperature was unchanged in the non-operated group of infertile patients with a left varicocele. Moreover, this reduction seems to be durable as temperature values were still decreased at 3 and 6 months after surgery. However, considering individual scrotal temperatures, only 51% of the patients in the operated group had an abnormal value before surgery.

Support : None.

PO 010

Prevalence of hypospermia and hyperspermia and their relationship with genital tract infection in tunisian infertile men

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Objective : The objective of our work was to determine the frequencies of hypospermia and hyperspermia and to establish their possible associations to other spermatic abnormalities and their possible relationship with the genital tract infection.

Design and Setting : Our retrospective study concerned 2332 spermiograms performed between 1995 and 2005 into the laboratory of Histology of Medicine University of Sfax, among patients consulting for couple infertility.

Materials and Methods : The spermiograms were carried out according to the standardized method of WHO. We distinguished three great groups according to the semen volume : Gn (normospermia), GH (hyperspermia) and Gh (hypospermia) ; from Gh we individualized one subgroup called Gh1 (severe hypospermie : volume<1ml). For all these groups, we determined the average values of semen parameters and compared them using the Student test; the frequencies of semen abnormalities (azoospermia, asthenospermia, necrospermia, oligospermia, leucocytospermia and teratospermia) were also determined and compared, using the Chi-2 test. The threshold of significance of p value was<0.05.

Results : The total prevalence of the hypospermia was 18.3%; severe hypospermia was found in 3.7% of cases. The frequency of the hyperspermia was only 6.8%. Many significant differences were found between Gh1, Gn and GH, mainly concerning motility, total spermatozoa count and vitality, which were lower in Gh and Gh1 groups, comparatively with Gn and GH groups, as well as the average rate of coiled tails and the frequencies of semen abnormalities; in fact the frequencies of asthenospermia, necrospermia, leucocytospermia, oligospermia and azoospermia were higher in Gh and Gh1, comparatively with Gn and GH. In addition, in Gh and Gh1, azoospermia was more frequently associated to abnormal pH than in Gn and GH ; thus basic pH (>8.5) was associated to azoospermia, respectively in 32.5% and 46.15% of Gh and Gh1 patients, versus 19.44% and 16.6% of Gn and GH patients; in the same way, azoospermia was associated to an acid pH in 4.6% and 15.38% of Gh and Gh1 patients, versus 0% of Gn and GH patients.

Conclusion : It seems that hypospermia is associated to an active genital infection responsible for many spermatic disturbances and for the appearance of biological inflammation signs (leucocytospermia). But, our results led to suggest that hyperspermia is not related to an evolutive genital tract infection, since the average values of the principal semen parameters, as well as the frequencies of semen abnormalities were comparable with those found in the normospermic group. We could also suggest in the light of these results that hyperspermia would be associated to a recent infection that did not yet induced semen quality disruption. In addition, we suggest that among azoospermic patients, hypospermia is related to a prostatic pathlogy (prostatitis with high pH in seminal plasma) than to a seminal vesicule deficiency (low pH in seminal plasma).

Prevalence of asymptomatic inflammatory prostatitis in young healthy men in Estonia

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Objective : The aim of our study was to determine the prevalence of asymptomatic inflammatory (NIH category IV) prostatitis in young healthy men in Estonia.

Materials and Methods : The study group consisted of 562 men (291 Estonians, 271 Russians) aged 16-25 years (mean age 18.8 years). Cytologic examination of their ejaculate (using Bryan-Leishman stained slides) was performed. In addition, all subjects were clinically examined for possible pathologies in genital region and basic semen parameters (volume, concentration and motility). Subjects with any clinical symptoms of inflammation were excluded.

Results : The prevalence of asymptomatic inflammatory prostatitis (>1 million WBC (white blood cells) per ml in sperm, according to WHO guidelines) was 6.0%, but when we used lower threshold suggested by our previous studies (>0.2 million WBC/ml), the prevalence was 19.0%. No difference between the two ethnic groups were found when seminal parameters and inflammatory markers were compared. In this study the preliminary analysis did not show any significant effect of leukocytospermia on sperm quality. We did not detect any seasonal differences in the prevalence of asymptomatic inflammatory prostatitis.

Conclusions : Asymptomatic inflammatory prostatitis is common among healthy young males, suggesting the need for further studies in order to investigate pathogenetic mechanisms of the disease. Our study also suggests that more attention should be paid to evaluation and treatment of asymptomatic inflammatory prostatitis in young men. Prevalence data we found should be taken into account when estimating the total prevalence of all forms of chronic prostatitis, both symptomatic and asymptomatic.

Support : The study was supported by Estonian Science Foundation (grant no 5701) and EU 6th FP project QLRT-2001-0291.

Genital tract infectious and inflammatory pathology and male infertility

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Objective : To assess the prevalence of genital tract infection and inflammation on male infertility and to elucidate the importance of both clinical, biological and ultrasonographic investigations in the diagnosis of the male chronic genital tract infection.

Design : Retrospective study.

Setting : Research Unit "male infertility", Habib Bourguiba Hospital, Sfax Tunisia and Laboratory of Histology and Embryology; Faculty of Medicine, Sfax Tunisia.

Materials and Methods : A total of 220 male partners of infertile couples were evaluated by the study of their medical file, biological investigations (semen analysis and culture) and ultrasonographic examination. Our cohort was subdivided into two groups : (G1, n= 49) included patients with genitourinary tract infection/inflammation; and (G2: n=171) included patients without genitourinary tract infection/inflammation. The presence of genital tract infection/inflammation was attested by the presence of bacteriospermia or leukocytospermia or by the existence of several clinical and/or ultrasonographic and/or other biological abnormal parameters. Statistical analysis was performed using Statview software. Frequencies were compared by the chi-square test and means values were compared by Student's unpaired t test. Level of significance was fixed at $p<0.05$.

Results : Genital infection and/or inflammation was detected in 22.3% in our patients (49/220). In history taking, epididymo-orchitis and urethral discharge were found with significantly higher prevalence in G1 (24.4% and 18.3%, respectively) than in G2 (5.8% and 5.8%, respectively) ; p values were 0.004 and 0.01, respectively. Urinary tract infection was detected in 16.3% of patients in G1 and 1.1% in G2 ($p<0.001$). Semen culture was negative in 38.8% of patients in G1. Semen parameters were altered in G1 with significant lower mean values of motility (total and rapid progressive spermatozoa), of vitality and of morphology (essentially tail defects) in G1 than in G2. Hypospermia was significantly more frequent in G1 than in G2 (32.5% vs 15.4%, $p=0.02$). The prevalence of leukocyospermia (>0,5millions/ml) was

also significantly higher in G1 compared with G2 (24.1% vs 2.6%, p =0.01). By ultrasonography examination, genital male tract abnormalitites (cysts, nodules) and accessory gland calcifications were founded more frequently in G1 (21.8% and 11.9%, respectively) than in G2 (2.8% and 0.7%, respectively), with respective p values : <0,001 and 0.003.

Conclusions : Our results show that genital male tract infection and/or inflammation occurs frequently in infertile patients. Negative semen culture has low sensitivity for discrimination between patients with and without infection. To establish the diagnosis of infection or silent genital tract inflammation, it would be necessary to confront the clinical, biological and ultrasonographic investigation results.

PO 012

Bacteria trigger production of reactive oxygen intermediates and lipid sperm membrane peroxidation in in vitro model

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Objective : The relationship between the presence of infectious factor in semen and sperm fertilizing potential remains to be actively studied. Microorganisms invasion results in the development of inflammatory process and is usually accompanied by the oxidative stress. As the role of particular groups and species of microbes invading, colonizing or infecting the male reproductive tract is still poorly understood, we aimed to assess an in vitro effect of the five most often isolated aerobic, anaerobic and atypic bacterial strains from semen of our infertile patients. These are : *Escherichia coli, Staphylococcus haemolyticus, Streptococcus oralis,* Bacteroides ureolyticus and Ureaplasma urealyticum. We have studied their influence on the level of reactive oxygen intermediates (ROI) generated in co-incubated suspensions of white blood cells (WBCs) and spermatozoa as well as quantitating sperm membrane malondialdehyde (MDA).

Design : An *in vitro* model of semen infection.

Materials and Methods : The venous blood samples were obtained from 10 healthy adults and WBCs were isolated using a density gradient centrifugation. Spermatozoa were isolated by swim-up technique ('swim-up' fraction) and discontinuous Percoll gradient (90% and 47% Percoll fractions) from 10 healthy, normozoospermic volunteers. The measurement of ROI secretion by WBCs previously incubated with bacterial strains before and after addition of spermatozoal fractions was determined by chemiluminescence in the presence of luminol. MDA concentrations were studied by using a high-performance liquid chromatography (HPLC) in sperm lysates after incubation of sperm with bacteria and/or WBCs.

Results : The presence of bacteria in co-incubated suspensions of sperm and WBCs was connected with reduced ROI scavenging potential of sperm, especially of spermatozoa with the best seminological parameters selected by swim-up procedure. An increase of detected MDA after incubation of sperm cells with bacterial strains was observed (which was a natural consequence of non neutralized ROI in co-incubated mixture of WBCs and spermatozoa) although no statistic difference was found. The presence of leukocytes generally was associated with elevated levels of MDA levels, in 'swim-up' fraction in particular, and the greatest effect was exerted by *B. ureolyticus* and *S. haemolyticus* ($p<0.01$ and $p<0.05$, respectively, in comparison to spermatozoa incubated with leukocytes only). Comparisons between MDA content in spermatozoal fractions with no leukocytes and after leukocytes incubation with selected bacterial strains exhibited significant differences when *Str. oralis, S. haemolyticus and U. urealyticum* were applied ($p<0.05$ and $p<0.01$).

Conclusions : The results obtained in this study constitute another evidence indicating that bacteria which invade, colonize or infect the male reproductive tract are important inducers of the oxidative stress in semen which may play a crucial role in male gamete dysfunction through the peroxidative damage of sperm membrane lipids. Our data support the hypothesis about cooperation between bacteria and WBCs in triggering both structural and functional defects in human germ cells. Possibly extended imbalance between pro-and antioxidative activities in semen, primarly caused by an infectious factor may lead to limited fertilizing ability of spermatozoa as a consequence of enhanced ROI reactivity with cell components.

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PO 013

Costimulatory molecules, chemokine receptors and proinflammatory cytokines in dendritic cell population in normal and chronically inflamed rat testis

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Dendritic cells (DC) are potent antigen presenting cells and presentation of self antigens by DC is likely to play an important role in the initiation of autoimmunity and its progression. Our recent characterization of testicular autoantigens in a model of chronic testicular inflammation, i.e. experimental autoimmune orchitis (EAO) prompted us to investigate the presence the DC in normal and EAO rat testis.

DC in the testes were quantified by immunohistochemistry using the DC specific antibodies Ox-62 and CD11c followed by stereological analysis. The number of DC in EAO testes (ca. 7x105/testis) increased significantly compared to adjuvant and untreated control rats (ca. 1x105/testis).

The activation state of the DC is crucial in determining the outcome of antigenic challenge viz the development of either T cell immunity or tolerance. To better understand the role of DC in testicular inflammation, we performed a detailed analysis of different maturation markers such as costimulatory molecules, chemokine receptors and cytokines.

We analyzed the expression of CD80, CD86 and MHC class II molecules on DC by flow cytometry in testicular single cell suspensions. Moreover, we have isolated testicular DC from adjuvant control and EAO adult rats by magnetic beads separation followed by FACS sorting and determined the expression of mRNAs for chemokine receptors (CCR2 and CCR7), IL10 and IL12.

Our preliminary results showed no significant differences in the expression of CD80, CD86 and MHC II between the investigated groups, but a significantly upregulated expression of CCR7 and a strong decrease of IL12 mRNA in the EAO group. The CCR2 mRNA level in EAO animals was not significantly changed comparing to adjuvant controls. These data suggest that the DC in EAO testis have already processed (auto)antigens and are in a status to migrate to the local nymph nodes for T cell activation. They are in a ready migratory state, but functionally immature.

Our data provide the first firm evidence for the existence of DC in the testis and in conjunction with the previous characterization of autoantigens a new tool for the investigation of the underlying causes of male factor immunological infertility.

PO 014

Uropathogenic E.coli but not commensale E.coli infection activates Toll-like receptor-4 dependent signaling pathways in rat testicular cells

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Immunological infertility such as infection, inflammation and autoimmunity account for at least 12-13% of all cases of male infertility. Uropathogenic Escherichia coli (UPEC) are the most common cause of urogenital tract infection, ultimately leading to infertility by germ cell loss. Toll-like receptor (TLR1-13) family recognize conserved microbial structures such as bacterial lipopolysaccharide, peptidoglycan and viral double-stranded RNA, and activate MyD dependent /independent signaling pathways that result in innate immune responses against microbial infections. All TLRs activate a common signaling pathway that culminates in the activation of NF-kappa B transcription factors as well as the mitogen-activated protein kinases (MAPKs) ERK, p38 and JNK.

The so-called "immunological privilege" of the testis is believed to arise from the need to prevent immune responses against the autoantigens of the meiotic and haploid germ cells, which first appear at the time of puberty, long after the establishment of self-tolerance. Paradoxically, the testis has an active defense mechanism which is illustrated by the obvious capacity for inflammatory responses to local and systemic infection. However, the testicular defence to infection, particularly to bacteria, are poorly defined on the molecular level.

In a first step, we investigated the mRNA expression pattern of TLRs in isolated testicular cells by RT-PCR. Most somatic and germ cell types expressed at least one TLR, with Sertoli

cells (SC) synthesizing some, peritubular cells (PTC) most, and testicular macrophages (TM) all TLRs. Isolated rat TM, SC, and PTC were infected with human uropathogenic E.coli (UPEC) and non-pathogenic commensale E.coli (NPEC). Following Western blot analysis TLR-4 protein levels increased 2h after UPEC infection in TM and SC. In contrast, TLR-4 levels in PTC increased only after 6h. Furthermore, we have also investigated the activation of the MAP Kinases (p38, JNK and ERK1/2) following UPEC infection in cultured testicular cells. Phospho-p38 levels were strongly enhanced after 30 min in TM, after 60 min in SC and after 120 min in PTC. ERK1/2 was phosphorylated transiently after 30 min in TM and at 60 min in SC, whereas PTC showed no activation. Only in SC JNK was activated following UPEC infection.

The transcription factor NF-kappa B plays a central role in immunological processes and several diseases. A primary level of control for NF-kappa B is through interactions with an inhibitor protein called I kappa B. Activation of NF-kappa B to move into the nucleus is controlled by the subsequent degradation of I kappa B, a process known to prevent apoptosis and induce proinflammatory cytokine production. TM and SC displayed no degradation of I kappa B alpha after UPEC infection, whereas in PTC I kappa B alpha degradation starts after 1h. This indicates that TM and SC are driven to apoptosis after UPEC infection, which is supported by preliminary results from apoptosis assays. Addition of NPEC to testicular cells showed no effect in any of the above mentioned experiments, but peritoneal macrophages as control were sensitive to both UPEC and NPEC. These results suggest that testicular cells are much more sensitive to UPEC infection than to other E. coli strains. Moreover, SC and TM are significantly more sensitive to UPEC infection than PTC and that apoptosis of SC can be an underlying cause for the observed loss of spermatogenesis during bacterial infection of the testis and excurrent ducts.

PO 015

Increasement of apoptotic spermatozoa in infertile men with inflammatory changes in seminal plasma

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Objective : It is more than likely that apoptotic sspermatozooas may hamper successful fertilisation. It is well known that inflammatory cytokines such as TNF-alpha may induce apoptosis.

So we investigated apoptotic spermatozooas in infertile men with inflammatory changes in the seminal plasma in comparison to infertile men with no signs of inflammatory changes by flowcytometric staining of Annexin-V.

Thereby we could demonstrate that spermatozooas from infertile men with inflammatory changes showed increased Annexin-V binding. In the subsequent ejaculate (1 hour after first ejaculation) we could demonstrate a further significant increase of apoptotic spermatozooas. Interestingly, we could detect a high number of apoptotic spermatozooas after swim-up. The rate of apoptotic spermatozooas was again higher within the inflammatory group of infertile men. Furthermore, we could demonstrate that apoptosis of spermatozooas was stable over period of up to three hour and did not change significantly in both group. This suggests that apoptotic spermatozooas in the swim-up fraction were motile and initialized the apoptotic process before the procedere.

Taken together our data demonstrate that infertile men with inflammatory changes in the seminal plasma show an increase rate of apoptotic spermatozooas which might hamper fertilisation. Furthermore our data might open a rational basis for antiinflammatory therapy to reduce apoptosis.

PO 016

Bacteriospermia and in vitro fertilization

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Objective and design : Bacteriospermia is frequent in the general male population like within men consulting for infertility. The purpose of in vitro fertilization, classical (IVF) or by intracytoplasmic sperm injection (ICSI), is to support the process of fertilization and early embryonic development within sterile culture media. The presence of bacteria in the seminal fluid could be a harmful factor in this context. In our work, we evaluated the effects of a significant bacteriospermia (<103 bacteria/mL), detected on the semen sample the day of oocyte puncture, on (i) sperm parameters, (ii) oocyte

fertilization rate, (ii) number and quality of the embryos obtained as well as (iv) pregnancy rate and evolution.

Materials and Methods : Our study was performed on 514 consecutive cycles of IVF or ICSI. A spermoculture was carried out on each semen sample collected the day of oocyte puncture. Volume, pH, sperm count as well as motility (a+b) constituted sperm parameters studied and were evaluated after liquefaction according to the World Health Organization. The number of oocytes collected, inseminated and/or injected and fertilized as well as the number and the quality of the embryos represented oocyte and embryonic parameters. The follow-up of the pregnancies thus obtained was also evaluated. Sperm preparation and the techniques of IVF and ICSI were standardized. Statistical analysis was carried out using software STAVIEW for WINDOWS (Abaccus Concept).

Results : Data collection relates to 514 cycles including 262 IVF (50.97%) and 252 ICSI (49.03%). Bacteriospermia was detected in 21.21% semen samples. Bacteria most frequently found were Ureaplasma urealyticum (33.9% of the positive cultures), followed by Chlamydia trachomatis (11%), Streptococci other than B (10.1%) and yeasts (1.8%). Among the various studied sperm parameters, only motility (a+b) appeared significantly decreased ($p<0,0125$) in men presenting a positive culture. No visible sign of infection was observed in IVF or ICSI culture dishes during this period. No significant difference was observed by comparing (i) the rates of fertilization in the infected group (49.68%) versus not infected (50.41%) as well as (ii) the number and the quality of the embryos obtained. On 125 pregnancies obtained, 25 (20%) were in the infected group but without finding any statistically significant difference in the rate of pregnancy between the two groups (infected 22.9% versus not infected 24.94%). The same applies to the frequency of pregnancy complications as well as fetal and neonatal pathologies.

Conclusion : Bacteriospermia does not constitute a priori a major factor susceptible to disturb the principal parameters of IVF or ICSI. The systematic practice of a spermoculture before Assisted Reproductive Technique (ART) procedure can be called in question. On the other hand, taking into account the probable role of certain bacteria in sperm motility alteration, it appears essential to carry out a spermoculture in any assessment of male infertility and this before proposing ART.

Simian Immunodeficiency Virus in the male macaque genital tract: target organs and cells during primary and chronic infection and impact of early-initiated antiretroviral treatment

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Objectives : Despite semen being the main vector of HIV dissemination, the sources of production of the viral particles contaminating this fluid remain unclear. Numerous studies have demonstrated compartmentalization of HIV strains between blood and semen, strongly suggesting viral production within the male genital tract (MGT). Moreover, men under antiretroviral treatment may still shed HIV in semen despite undetectable blood viremia (reviewed in (1, 2)). In this context, determining the susceptibility of the MGT to HIV infection and the impact of antiretroviral treatments within this body site is of prime importance. Our study used SIV-infected macaques as an animal model to research this issue.

Methods : Macaques inoculated intravenously with SIVmac251 were sacrificed either during primo-infection at 14 days post-inoculation (n=4) or during the chronic phase (n=7). In addition, some animals were submitted to antiretroviral treatment initiated either 4h (n=4) or 7 days post-infection (n=3) for a 2 week period before sacrifice. Testes, prostates, epididymis and seminal vesicles were recovered and the presence of SIV RNA and DNA determined in nested RT-PCR and PCR, respectively. Immunohistochemistry and in situ hybridization were performed to appreciate the distribution of immune and SIV infected cells.

Results : In chronic and primary-infected untreated monkeys, viral RNA and DNA were detected in all the genital organs, with a frequency positively correlated with plasma viral load. Notably, elevated levels of detection of SIV nucleic acids were observed during primo-infection. Infection was consistently more frequent in the accessory glands than in the testes. The accessory glands often presented varying degrees of HLA-DR+ inflammatory infiltrates, observed as early as 14 days post-infection, and mainly composed of cytotoxic T lymphocytes (CD3+TIA-1+). Using combined *in situ* hybridization for SIV gag RNA and immunohistochemistry for specific cell markers, we demonstrated the presence of SIV RNA co-localizing with CD68+ myeloid cell in the prostate of chronic and primary infected macaques. Two out of four animals treated as early as 4h post-infection and four out of four animals treated 7 days post-infection still displayed SIV

DNA in the accessory glands, in spite of a drastic decrease of the plasma viral load.

Conclusions : SIV infection of the male genital tract occurs early, the virus being detected as soon as 14 days post-infection. Infection of the MGT is associated with high blood viremia and triggers an inflammatory response. Early initiated treatment applied for a short duration does not prevent the viral spread to the male genital organs.

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PO 018

Azoospermia and normal follicle stimulating hormone (FSH) levels - role of α-glucosidase as discriminator between patients with or without obstruction

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Objective : Although α-glucosidase determination is included in the WHO Handbook for Semen Analysis (1), its value in the infertility workup is still under debate (2). We reinvestigated the diagnostic power of α-glucosidase to distinguish between obstructive (OA) and non-obstructive azoospermia (NOA).

Design : Retrospective analysis of patients' data retrieved from the institute's database Androbase© (3).

Materials and Methods : 164 healthy volunteers with normal sperm concentration (> 20 x 10^6/ml) formed the control group. The group with proven OA consisted of 86 patients with either diagnosis of vasectomy (n = 55) or CBAVD (n = 31). 79 patients with azoospermia and normal FSH levels (≤ 7 U/l) and no apparent clinical reason for their azoospermia (such as Klinefelter syndrome, post-chemotherapy, tumours, etc.) were subdivided into two study groups according to an ·α–glucosidase threshold level derived from its frequency distribution in the control group. All men underwent routine andrological workup. Testicular biopsies were available from 32 patients.

Results : The 2.5-percentile (16 mU/ejaculate) of α-glucosidase levels in the control group was chosen as the cut-off value to categorise the study group. 33 patients had α-glucosidase levels < 16 mU/ejac. (suspected OA) and 46 were above this threshold (suspected NOA). The suspected OA group was indistinguishable from the group with proven OA in all relevant parameters evaluated. In contrast, the group with suspected NOA differed significantly from the proven OA group in regard to semen volume, fructose and α-glucosidase, which were higher, while testicular volume and age were lower. No differences in hormone levels were found except that the control group had higher LH and lower FSH levels. All patients of the suspected OA group with available histology had elongated spermatids in their biopsy (16/16) while in the suspected NOA group several patients with spermatogenic arrest were identified (6/16 ; 10 had elongated spermatids) giving a specificity of 100% (pos. predictive value : 100%) and sensitivity of 62% (neg. predictive value : 38%) for an α-glucosidase level of 16 mU/ejac.

Conclusions : The α-glucosidase level of 16 mU/ejac., which is slightly lower than the WHO cut-off level of 20 mU/ejac. derived from proven fathers, provides a discriminator to help counselling patients with azoospermia and normal FSH levels with regard to the chance of finding sperm in a biopsy (specificity : 100%). However, the sensitivity of 62% is rather poor. Fructose and zinc do not improve the diagnosis.

Support : No external support.

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Measurement of steroid hormones concentration in peripheral and spermatic blood in infertile patients with non-obstructive azoospermia : A prospective comparative study

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Objective and design : The serum levels of steroid hormones (testosterone, estradiol) were compared between spermatic vein blood and peripheral blood, in patients with non obstructive azoospermia (NOA), according to the results of surgical sperm retrieval. The aim of our study was to identify predictive factors of successful testicular sperm recovery in patients with non obstructive azoospermia.

Materials and Methods : A Prospective and comparative study (supported by a Rouen Hospital Grant and approved by the local ethical committee) was performed in a population of of 30 patients with NOA. For each subject who underwent testicular sperm extraction, serum levels of testosterone (T) and estradiol (E2) were determined in spermatic vein blood (Ts and E2s) and in peripheral blood (Tp and E2p). Differences in hormone levels between the two groups of patients with either successful or unsuccessful sperm recovery were analysed using non parametric variance analysis and Mann-Whitney U-test.

Results : Data are presented as the mean values with ranges.

Sperm Extraction	Ts (ng/ml)	E2s (pg/ml)	Tp (ng/ml)	E2p (pg/ml)	E2s / Ts
Successful (43%)	496,65 (9,45-1880)	1059,23 (28-5300)	4,55 (2,0-9,9)	21,08 (9,0-30,0)	0,002 ***a*** (0,001-0,005)
Unsuccessful (57%)	358,18 (12-1126)	1310,88 (28-4850)	4,7 (2,0-8,0)	26,47 (16,0-39,0)	0,004 ***a*** (0,001-0,009)

Conclusion : The ratio E2s/Ts appears significantly increased in the group with failure of testicular sperm retrieval (p=0,018). These data suggest that aromatase activity is higher when haploid germ cells are absent of the testis. The mechanisms of action of estrogens in the spermatogenesis remain to be clarified, especially with studies using another marker like tissue marker (cytochrome P450 aromatase activity, aromatase gene *(CYP 19)* expression).

A long term quality control for a serum inhibin B assay

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Objective : Inhibin B in serum is a well-established marker for Sertoli cell function and spermatogenesis. The present study evaluates the reproducibility of an enzyme linked immunosorbant assay (ELISA) for inhibin B.

Materials and Methods : Two pooled blood sera with low (PL) and high activities of inhibin B, were included in each of 57 assays for inhibin B (Serotec, Oxford, UK) and the assay was performed at our laboratory according to manufacturer's instructions.

Pooled serum samples were processed in duplo allowing for the calculation of inter assay co-efficient of variation (CV) and the averages of the two readings in the different assays was used to calculate the inter assay CV. In total data on 3 different pairs of pools used in 57 runs for the period 1998-2002 was available for analysis.

Results : The average intra-assay coefficient of variation (CV) for all the inhibin B runs performed at our laboratory was 6.9 % for the serum pools with low inhibin B levels (PL) and 4.4 % for those pools with high inhibin B activities (PH). The inter-assay CV ranged from 19 to 34 % (see table 1).

Table 1 : Intra- and inter-assay coefficient of variation (CV) for inhibin B measurement.

	PL1	PH 1	PL 2	PH 2	PL 3	PH 3
Number of runs	24	24	22	22	11	11
Average intra-assay CV (%)	6.8	4.0	8.0	5.6	5.2	2.7
Inter-assay CV (%)	24.3	19.2	20.0	34.0	28.0	30.2
Average inhibin B (ng/dl)	84.5	193.7	82.4	269.1	63.8	222.2

Conclusions : The high interassay variability might be explained by the use of different batches of inhibin B kits over the period of 5 years. The within batch interassay CVs is much lower (data not shown). The high inter-assay CVs over a long period of time indicates, however, that comparing results from different studies where different batches of inhibin B kits were used is not recommended. **Support :** none

PO 021

Inhibin B as a predictor before testicular sperm extraction – valuable completion or cost factor ?

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Introduction : Inhibin B as a predictive marker for a successful testicular sperm extraction (TESE) is discussed controversely in the literature. The aim of this study was to evaluate the predictive value of Inhibin B as a reliable marker for spermatogenesis in patients (pats.) with nonobstructive azoospermia (NOA).

Materials and Methods : In 78 pats. (33±4.8 years) undergoing TESE the following parameters were evaluated: (a) testicular volume; (b) serum follicle hormone (FSH) and (c) serum Inhibin B level. These parameters were correlated to the TESE-results (recovery of spermatozoa).

Results : In 49 of the 78 pats. (62%) spermatozoa were successfully retrieved (TESE-positive). The Serum inhibin B level was 103,5±12,5 pg/ml in the TESE-positive group. Compared to a control group (healthy probands) there was a significant difference regarding serum Inhibin-B levels between the TESE positive group and control group (103.5 vs. 183.6 pg/ml; $p<0.05$). We observed also a statistically significant difference of the serum Inhibin-B levels between controls and pats. with no evidence of spermatozoa in TESE (92.8 vs. 183.6 pg/ml; $p<0.05$). No statistically significant difference exists for the Serum Inhibin B levels in TESE-positive and TESE-negative pats. ($p>0.05$).

In contrast, a significant difference in serum FSH was evident. In TESE-positive pats. the serum-FSH level was 14.6±1.9 IE/l, in TESE-negative pats. 20.3±1.9 IE/l ($p<0.05$).

In pats. with normal serum FSH 82% of sperm retrieval attempts were successful, whereas in the group with pathologic serum FSH only in 42% of attempts sperms were retrieved ($p<0.05$). Additionally, spermatozoa recovery correlated also with testicular volume ($p<0.05$).

Conclusions : Serum FSH and testicular volume are valuable marker to estimate the expecting results of a testicular sperm extraction. In our hands Serum inhibin-B wasn't able to give further information predicting the possible evidence of spermatozoa.

No differences in the serum Inhibin B levels were observed in TESE-positive and TESE-negative pats.. But TESE-positive and TESE-negative pats. had significant lower serum Inhibin B levels compared to the control group, $p<0.05$.

In pats. with normal serum FSH 82% of sperm retrieval attempts were successful, 27/6 vs 22/23, $p<0.05$.

In TESE-positive pats. the serum-FSH level was with 14.6±1.9 IE/l significantly lower compared toTESE-negative pats. 20.3±1.9 IE/l ($p<0.05$).

PO 022

Testicular Fine Needle Aspiration Biopsy in the investigation of the subfertile male with azoospermia and severe oligo-terato-asthenospermia

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Objective : To describe our experience with testicular Fine Needle Aspiration Biopsy (FNA) in the investigation of subfertile men with azoospermia and severe oligo-astheno-teratospermia (OAT).

Patients and Methods : From 1999 to 2005, 1087 subfertile men were studied at the outpatient clinics of a referral Andrology center in Northern Greece. All men underwent clinical, sperm, basal and dynamic hormonal evaluation. Genetic studies were applied as appropriate. Azoospermia and severe OAT were found in 78 (7.9%) and 27 (2.7%) men, respectively. Testicular FNA underwent 99 (94.2%) of these men. In addition, 15 men underwent Testicular Sperm Extraction (TESE) at a time later than FNA.

Results : Etiological diagnosis of the studied men were: Idiopathic Non-Obstructive Azoospermia (INOA) with or without Late-Onset Hypogonadism (LOH) (n = 51, 52%), crytporchidism (n = 14, 14%), congenital agenesis of vasa deferentia (n = 8, 8%), obstructive azoospermia (n = 7, 7%), varicocele (n = 13, 13%), Klinefelter syndrome (n = 2, 2%), and other causes (n = 4, 4%). Overall, the cytological diagnoses were : normal spermatogenesis (n = 18, 19%), moderate hypospermatogenesis (n = 16, 17%), severe hypospermatogenesis (n = 24, 24%), incomplete maturation arrest (n = 2, 2%), complete maturation arrest (n = 14, 14%),

complete Sertoli-Cell only Syndrome (SCOS) (n = 22, 22%), and insufficient sample (n = 3, 2%). The cytological diagnoses in the subgroup of 51 men with INOA were: normal spermatogenesis (n = 1, 2%), moderate hypospermatogenesis (n = 7, 14%), severe hypospermatogenesis (n = 18, 35%), incomplete maturation arrest (n = 2, 4%), complete maturation arrest (n = 9, 18%), complete Sertoli-Cell only Syndrome (SCOS) (n = 13, 25%), and insufficient sample (n = 1, 2%). Finally, during the TESE procedure, sperm was found in 7 men whereas not found in the remaining 8 men ; of them, the positive result was predicted during the FNA procedure in 6 men (Positive Predictive Value – PPV: 86%) and the negative result was predicted in 6 men (Negative Predictive Value – NPV: 75%).

Conclusion : FNA plays an important role in both the diagnostic and therapeutic approach of subfertile man. In the diagnostic approach, a single testicular FNA according to our results has 51% possibility of detecting sperm in subfertile men with INOA. Aspirated spermatozoa can then be used for assisted reproduction techniques, such as Intra-Cytoplasmic Sperm Injection (ICSI). In the therapeutic approach, FNA has 86% PPV and 75% NPV of predicting TESE results, further facilitating the ICSI procedure.

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PO 023

Presence and quality of testicular spermatozoa in azoospermic men according to region of their residence in Slovenia

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Objective : Little is known about the effect of geographical factors on the appearance and type of azoospermia, and manifestation of testicular cancer.

Design : The aim of this preliminary study was to compare the presence and quality (motility) of testicular spermatozoa, and outcome of ICSI in patients with azoospermia according to 8 Slovenian geographical regions of their residence. Additionally, the incidence of testicular cancer in their medical history was compared among the regions.

Material and Methods : In this study 192 diagnostic testicular biopsies from 192 infertile men with azoospermia attending our Department were included. Each biopsy specimen was checked for the presence of spermatozoa. In patients with spermatozoa sperm motilility was evaluated (motile and non-motile spermatozoa). The testicular tissue was cryopreserved for later in vitro fertilization. The men were divided in 8 groups by the geographical region of their residence: I. Capital Ljubljana (n =71), II. Region of _tajerska (n = 30), III. Region of Gorenjska (n = 25), IV. Region of Primorska (n = 20), V. Region of Dolenjska (n = 13), VI. Region of Koro_ka (n = 12), VII. Region of Severna Primorska (n = 9), VIII. others – Idrijsko-Cerkljansko, Posavje, Zasavje (n = 12). All groups of men were statistically compared according to the presence and motility of testicular spermatozoa, ICSI outcome, and the incidence of testicular cancer in their medical history.

Results : The mean male age was 36 years (min. 25 – max. 56 years) with no statistically significant difference among regions. There was no statistically significant difference among regions in the proportion of men with testicular sperms (I. 65%, II. 57%, III. 72%, V. 77%, VI. 67%, VIII. 70%), except for the Primorska region (IV) : a significantly lower proportion of men (35% ; $P < 0.05$) due to a higher incidence of Sertoli Cell Only Syndrome and maturation arrest. Also a higher incidence of testicular cancer in the medical history was registered in the men from the region of Primorska than from other regions (15% vs 4%, $P < 0.05$). We found no significant differences in the proportion of men with motile sperms. In researched group of men 68 ICSI cycles were performed and 16 pregnancies (33.5% pregnancy rate per cycle) resulted (I. 23%, II. 27%, III. 37.5%, V. 25%, VI. 20%, VIII. 25%), none to a couple from the Primorska region (0%). Ten children were born (7 girls and 3 boys), 2 pregnancies are still on-going, and 4 pregnancies ended in a spontaneous abortion.

Conclusions : In this preliminary study, performed in a small group of azoospermic men, we found that the Primorska region (towns Koper, Piran, Portoro_, Izola, Ankaran) situated at the coast of the Northern Adriatic Sea (Gulf of Trieste, Gulf of Koper, Gulf of Piran) shows negative effects on spermatogenesis and on incidence of testicular cancer. This study will be extended to a bigger group of azoospermic men to exclude possible bias (like genetic abnormalities and cryptorchidism), and to evaluate the possible environmental effects of the Primorska region.

Support : We would like to thank to Mr. Matjaz Klun, B. Sc., lawyer, Ministry of the Environment and Spatial Planning of Republic of Slovenia and to Ms. Mojca Pirc, B. Sc.

PO 024

Recombinant FSH for the treatment of idiopathic infertility

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Introduction : Several experimental studies have demonstrated the key role played by FSH in normal spermatogenesis. Our objective was to analyze the results of the treatment with recombinant FSH in normogonadotropic patients with severe idiopathic olygospermia.

Patients and Methods : 50 males with idiopathic infertility, aged between 20 and 43 years old where included, based on the following criteria : severe olygospermia, infertility diagnosed at least 3 years ago, normal cariotype, normal ultrasonographic aspect of the testis, no associated pathology. Hormones involved in the reproductive function were measured by immunometry and only patients with normal hormone levels were included. Circadian patterns of urinary gonadotropes were determined in controls and infertile patients. In all patients, the free androgen index and the ratio FSH/LH were determined. 10 men with normal spermograms which conceived in the last 12 months represented the controls. All patients where treated for 20 weeks with, recombinant FSH, 50IU, 3 times a week. In those with low FAI testosterone, undecanoate 40mg/day was added. After 10 weeks of treatment, patients were reevaluated, using a spermogram and a hormonal profile.

Results : In all cases, circadian patterns of gonadotropes are very close, characterized by a synchronization of the secretion of the 2 hormones. After the treatment with recombinant FSH, the mean concentration of spermatozoids increased four times. 42% of the patients have normal spermatic parameters, and a mean FSH/LH ratio of 1 at 20 weeks after the treatment. 52% of the patients induced pregnancy after the treatment. In 8% cases, pregnancies terminated with first trimester spontaneous abortions.

Conclusions : Our study proves the efficacy of the treatment with recombinant FSH in males suffering from idiopathic infertility. It also proves the necessity of synchronous secretion of gonadotropic hormones for a normal spermatogenesis.

PO 025

Fertility in men with renal insufficiency before and after transplantation

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Objective : To determine the status of fertility in men with renal insufficiency and to analyze the effect of transplantation on fertility.

Design : self-administrated questionnaire.

Materials and Methods : A series of 239 renal transplanted men were evaluated through a self-administrated questionnaire focusing on fertility. Questions concerned the period before hemodialysis, after hemodialysis, and after transplantation.

Results : Before hemodialysis, 173 men tried to have children, 163 succeeded (94.2%) and 10 patients did not succeed (5.8%).

After the beginning of hemodialysis, 14 patients tried to have children. Among them 4 patients succeeded (29%) and 10 patients did not succeed (71%).

After transplantation, 51 patients tried to father and 27 succeeded (53%). Andrological evaluation of the 24 infertile men revealed erectile dysfunction in 7 cases and ejaculation disorders in 6 cases. Time elapsed between transplantation and the onset of pregnancy was 4 +/- 3 years (1-15). Among them, 8 were hemodialysed due to graft function deterioration. Among the 43 remaining patients, the fertility rate was 63%.

Conclusion : fertility decrease dramatically in hemodialysed patients from 94% to 29%. Renal transplantation is a way to recover fertility up to 63%.

PO 026

Modeling of the upper range of scrotal temperature in a driving situation

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Objectives : Elevation of testicular or scrotal temperature, either isolated or associated with a temperature rise of the whole body, leads to functional disturbances reflected in decreased quality and quantity of the gametes produced. This has been demonstrated in experimental studies in fertile men and observed in studies of infertile populations. However, determination of maximum scrotal temperature, which we will call the "upper range" is still an open question. We sought the upper range by recording the maximum reached during the temporal gradient of right and left scrotal temperature and rectal temperature under the particular environmental conditions of car driving.

Materials and Methods : Eight fertile male volunteers (20 to 48 years old) were studied after a continuous uninterrupted period of 180 minutes of driving a motor vehicle in a sitting position. History taking and clinical examination showed that all men were free of any medical, surgical or occupational histories, with no clinical varicocele or testicular hypotrophy or atrophy. Testicular volumes were measured with callipers. Temperatures were measured every 2 minutes with an AM-7001 precision skin thermometer (reading accuracy 0.1%, precision 0.03°C and sensor type K, Anritsu Meter Co., Tokyo, Japan).

Mathematical and statistical analysis :

1) Modeling of the physiological parameters which play a part in determining the upper range of temperature:

Adaptive ability of the scrotum: calculation of immediate and mean speeds of temperature change.

Mechanisms of scrotal thermoregulation: time to initiation of thermoregulation.

Scrotal sweating : computing of a periodogram.

2) Stepwise regression method : model selection and Akaike criterion.

Results and Conclusion : Under the same conditions, the upper range of scrotal temperature differs from one individual to another. This upper range is essentially explained by two factors: the initial values of scrotal temperature (physiological values with no major thermal constraint), and the ability of the scrotum to adapt in abnormal conditions such as driving.

Support : None.

PO 027

Complex molecular analysis of genetic factors in Russian infertile men

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Objective : To evaluate the frequency of common genetic infertile factors in the cohort of Russian men with azoospermia or severe oligozoospermia.

Design : Prospective study.

Materials and Methods : We have examined a cohort of 281 Russian infertile men. In all patients azoospermia or severe oligozoospermia has been diagnosed by sperm analysis (WHO, 1999). The individuals with chromosome abnormalities were excluded from study. Molecular investigation was carried out on leucocytes DNA. All DNA samples were examined for complete and partial AZF deletions. Detection of Y-chromosome microdeletions was carried out according to Laboratory guidelines (Simoni et al., 1999) by analyzing of recommended STSs plus sY615 in multiplex PCR. The DNA samples with no deletions have been tested on partial AZFc deletions in multiplex PCR, which included following STSs: sY142, sY1197, sY1192, sY1291, sY1206, and sY1125. Furthermore, eleven common CFTR gene mutations (del21kb, delF508, delI507, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, G542X, W1282X, N1303K), IVS8 poly (T) variants, and androgen receptor (AR) CAG-repeat polymorphisms were analyzed in 98 and 68 patients, respectively.

Results : Complete AZF deletions (AZFa – 2; AZFb+c – 2, AZFc/ b2/b4 – 24) have been found in about 10% of all examined chromosomes. Also thirty-three partial AZFc deletions (gr/gr deletions – 7, b2/b3 deletions – 22, del sY1197 – 3, del sY1197 and sY142 – 1) have been revealed in 11.7% patients (13% men with no complete AZF-deletions). Nine heterozygous CFTR mutations (ΔF508/- – 3, W1282X/- – 2, 2143delT/- – 2, del21kb/- – 1, and 2184insA/- – 1) have been found in 9 of 98 (9.2%) examined. 5T allele polymorphisms (7T/5T – 10, 9T/5T – 2) were found in 12.3% infertile men, including one patient with heterozygous ΔF508 mutation. In one case we have found determined revealed complete AZFc deletion (b2/b4) and IVS8-5T variant in heterozygous state simultaneously.

CAG repeat length in infertile men varied from 15 to 33 (median 22, mean 22.99), moreover 19% examined had increased CAG-repeat numbers (n≥26).

Conclusions : In Russian azoospermic or severe oligozoospermic men the frequencies of complete and partial AZF-deletions are nearly equal. The results of this study displayed that investigated genetic factors have high prevalence in severe male infertility. In this situation complex genetic examination is more preferred.

Support : none.

PO 028

Role of the X-chromosome linked, testis-specific TAF7L gene in gonadal function and spermatogenic failure

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Objective : The precise temporal and partial expressions of specific transcription regulation factors (TRF) have long been considered essential for proper execution of spermatogenesis. Recently, mammals have been speculated to have evolved more specialised TRF genes. In human the X-linked, testis-specific gene TAF7L gene may be obligatory for maintenance of spermatogenesis. In this study, we attempted to investigate the possible role of TAF7L gene in testicular function and spermatogenic failure.

Design : The study is a case-control retrospective study.

Materials and Methods : Sixteen carefully selected infertile males with consistent, non-obstructive azoospermia without elongated spermatids in testis biopsy (available in some) and with normal serum follicle stimulating hormone levels were selected for this study. Twenty age-matched men with normal spermatogenesis with the same ethnic background (Caucasian) were recruited as controls. DNA extracted from EDTA whole blood was screened for sequence changes in the coding regions and part of the flanking introns of the TAF7L gene by direct sequencing. Amino acid sequence was compared to the NCBI standard sequence.

(Hyprelink"http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=Nucleotide&dopt=GenBank&val=BC043391"BC043391).

Semen analysis and hormone evaluation were performed.

Results : Five alterations, consisting of three exonic changes in the nucleotides sequence of exon 9, 10, 13, and two changes in the flanking introns of exon 8 and 13, with concomitant changes in amino acid sequence were observed in 6 patients. Most of these alterations were also found in 8 controls with the exception of changes in exon 13. Though none of these changes were previously described in NCBI database, some are described in a recent publication (1). There was no association or relationship observed with reproductive hormones.

Conclusions : The alterations in the cDNA sequence observed are probably polymorphisms. However, the haplotype observed in exon 10 and the changes in the intron of exon 8 appears novel. We report for the first time that these alterations in nucleotide sequence of TAF7L gene are not associated with gonadal dysfunction.

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1) Stuoffs K., Willems A., Lissens W., Tournaye H., Van Streirteghem A., Liebaers I. : The role of the testis-specific gene hTAFL in the aetiology of male infertility. Mol. Hum. Reprod., 2006, 12 : 263-267.

PO 029

DAZ (Delected in AZoospermia) gene: an evidence of Y chromosome evolution

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Objective : Better understanding the heterogeneity and instability of the AZF loci on the human Y chromosome from fertile and infertile individuals, to determine which cases could present higher predisposition for a quick evolution since oligozoospermia to azoospermia. Check the existence of an association between the Y-haplogroups and the DAZ gene partial deletions.

Design : The human Y chromosome is strictly paternally inherited and, in most of its length, does not engage in pairing and crossing over during meiosis. The DAZ gene, candidate to fertility factor, lies within human Y chromosome's AZFc region (Azoospermic Factor) whose deletion is a common cause of spermatogenic failure. The Y single-nucleotide polymorphisms (Y-SNPs) on the non-recombining Y region (NRY) are well characterized and permit the construction of a unique phylogeny of haplogroups. DAZ haplotypes were defined using Single Nucleotide Variants (SNVs)/Sequence Tagged-Site (STSs) markers to distinguish the four copies of the gene (1). The variation of ten Y-chromosome Short Tandem Repeat (STRs) was used to determine the coalescence age of DAZ haplotypes in a comparable time frame similar to that of SNP haplogroups.

Materials and Methods : DNA samples from 97 azoospermic men ranging from Sertoli-cell-only syndrome (SO) to maturation arrest (MA) and hipospermatogenesis (HP) were analysed. A control group of 91 fertile men was also studied. All males involved in this study gave an informed consent, following local ethical guidelines. The Y-chromosome typing (24 SNPs and 10 STRs) was assayed and the haplogroup nomenclature and phylogeny used was the one proposed by the Y-chromosome Consortium (2002). DAZ haplotypes were determined by analysis of 6 DAZ-SNVs (I-VI) and 2 DAZ-STSs (DAZ-RRM3 and Y-DAZ3) (1). The coalescence time within each DAZ haplotype was defined using 10 STR (2).

Results : An association between DAZ haplotypes and Y chromosome haplogroups was found and our data shows that the DAZ gene is not under selective constraints and its evolution depends only on the mutation rate. The same variants were common to fertile and infertile men, although particular DAZ partial deletions occurred only in infertile ones, suggesting that infertility is due to gene conversion mechanisms or other chromosomal rearrangements (3).

Conclusion : As DAZ deletions might be a polymorphic event associated to a specific haplogroup or an individual cause of infertility, DAZ partial deletions should only be used as a tool for infertility diagnosis when analysed in combination with Y-haplogroup determinations. The possibility that a mutation defining a haplogroup could be something more than a single mutation - for instance, being associated with Y chromosome rearrangements - remains open for debate.

Support : (1) Fernandes et al. 2002 ; (2) Zhivotovsky et al. 2004 ; (3) Fernandes et al. 2006.

Lack of association of Y chromosome gr/gr deletions with spermatogenic failure

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Objective : Complete deletions of the AZFc region are associated with spermatogenic failure and occur with a frequency of 0.00025% in the general male population. Recently, partial deletions of AZFc have been reported to be a risk factor for developing infertility.

Design : Here we report our latest data on the investigation of partial AZFc deletions (gr/gr) in both control (fertile/normospermic men n=186) and case (spermatogenic failure, n=329) cohorts from the Tenon Hospital in Paris.

Materials and Methods : DNA from each patient was tested for partial AZFc deletions using a series of Sequence Tagged Sites markers from the region. In addition, each sample was tested with informative polymorphisms to distinguish between the DAZ1/2 and DAZ3/4 gene clusters as well as CDY1a and CDY1b. The haplogroup of each individual was defined using 7 binary markers. Finally the samples were tested for DAZ copy number using dosage assays.

Results : The incidence of gr/gr deletions was similar in each group: 11/329 (3.3 %) spermatogenic failure, 6/186 (3.2 %) control samples. Using polymorphic markers for DAZ1/2, DAZ3/4 and CDY1a /CDY1b, we did not observe a correlation between absence/presence of these genes and the fertility status. Y chromosome haplogroup distribution was similar in each group. Of the 11 cases with the gr/gr deletion, 10 showed the presence of 2 copies of DAZ, whilst one (control male) had 4 copies of DAZ.

Conclusions : Taken together our data indicate that partial AZFc deletions are relatively common compared to AZF deletion. Using available markers and gene dosage assays we could not distinguish between the case and control groups. These data are consistent with the hypothesis that the gr/gr deletion is an inconsequential polymorphism, at least in an ethnically heterogeneous Parisian population. Further studies are underway using fiber-FISH technology to understand the molecular nature of these deletions in both study groups.

PO 031

Fine molecular characterization of a new genetic risk factor for oligo/azoospermia

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Objective : "gr/gr" deletion, a newly identified partial deletion of the AZFc region of the Y chromosome, has been reported as a new risk factor for male infertility. Subsequent studies questioned the association of this genetic anomaly with infertility.

Materials and Methods : A part from our study and the original report in which all deletions were confirmed by quantitative assays, the detection of gr/gr deletions is currently based on a sequence tagged site (STS) +/- PCR method. The confirmation of the deletions PCR results is fundamental since rearrangements in this region may modify the primer annealing sequences leading to false deletions. Moreover, duplications identifiable only by dosage analysis may restore normal gene dosage and thus be compensate for a partial deletion. Using a combined method based on STS +/- followed by quantitative gene dosage and qualitative assays – to confirm the deletion and to define the number and the type of deleted DAZ and CDY genes – we identified three different gr/gr deletion patterns. The heterogeneity of the phenotypes associated to gr/gr deletions maybe therefore related to different gr/gr deletion subtypes.

Results : The aim of this study was to further increase the size of our study population and to provide a tool for the distinction between pathogenic and neutral types of gr/gr deletions by fine molecular characterization of the deletions and Y haplogroup definition. We analyzed a total of 557 patients and 364 controls. The frequency of gr/gr deletions in patients was significantly different from the normospermic controls (4,1% versus 0,2%, respectively $p<0,001$; OR=9,7 with CI 95% 1,4-66,2). Furthermore we have calculated how many subjects, identified with gr/gr deletion by the first step PCR +/- method were not confirmed by gene dosage and sequence family variant analysis. We observed a false deletion rate of 18% (6/33) indicating that STS +/- method alone does not provide reliable results.

Conclusions : In conclusion, we confirmed on a large group (total n=921) of subjects that gr/gr deletion is a risk factor for reduced sperm count in the Italian population. The role of gr/gr deletions as risk factor for reduced sperm count is currently debated and it maybe related to different factors: ethnic origin (Y background), lack of confirmation of results by dosage analysis, analysis of control groups with unknown sperm counts. We propose that different Y chromosome backgrounds and subtypes may confer protection against the deleterious effect of gr/gr deletion and thus a simple STS based method is insufficient to provide clinically relevant information.

PO 032

The impact of androgen receptor polymorphism and parental ethnicity on semen quality in young men from Latvia

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Objective : Recent studies of young men from the general population have demonstrated geographical and ethnical differences in semen quality with high sperm counts in the Baltic countries. The aim of this study was to investigate whether semen quality in Latvian men might be related to the maternally derived polymorphisms in the androgen receptor (AR) gene or rather to the ethnicity of the fathers.

Materials and Methods : In total 119 military conscripts from Latvia were included in the study. One hundred and fourteen men with available AR gene polymorphism data were divided into two groups according to their mother's ethnicity: men with Latvian mothers (n=83) and those with non-Latvian mothers (n=31). Regions outside of Latvia from which the parents originated included Russia (n=33), Ukraine (n=9), and Byelorussia (n=6). To assess the impact of father's ethnicity, men were additionally divided into two groups according to their father's ethnicity : men with Latvian fathers (n=77) and men with non-Latvian fathers (n=37). The influence of ethnic origin and AR polymorphisms on sperm concentration, total sperm count, semen volume and proportion of progressively motile sperms was analyzed in general linear regression models adjusted for the abstinence time. Influence of the mother's ethnicity was additionally adjusted for father's ethnicity, and vice versa.

Semen analysis was performed according to WHO recommendations. DNA was extracted from leukocytes and the numbers of CAG and GGN repeats obtained by direct sequencing Material was available for sequencing of the CAG and GGN tracts in 114/119 (96%) and 99/119 (83%) of the men, respectively.

Results : Men with Latvian mothers had borderline significantly shorter CAG repeat length (21.6±2.9) as compared with those with non-Latvian mothers (22.9±3.2, p=0.05). Sperm concentration did not differ significantly between these two groups (76±59 and 70±52, p=0.9, respectively). In contrary, father's ethnicity exhibited significance for sperm concentration and total sperm count, with higher numbers in men with Latvian fathers (n=77) as compared to men with non-Latvian fathers (n=37) (80±61 vs 62±48, p=0.035, for sperm concentration; 225.7±209 vs 158.4±134.4, p=0.002, for total sperm count, respectively). CAG repeat length was associated with sperm concentration in the whole study population: men with high sperm concentration (≥50 x 106/mL, n=70) had shorter CAG repeat length (21.4±2.9) than those with low sperm concentrations (<50 x 106/mL, n=44): 22.8±3.1, p=0.03. GGN repeat length, in turn, correlated only with semen volume: men with GGN>23 had higher semen volume (3.2±2.1) as compared with those with GGN=23 (2.6±1.3, p=0.04) and GGN<23 (2.0±1.2, p=0.006).

Conclusions : In Latvian men the origin of the father seems to play a more important role for sperm concentration than the maternally derived AR polymorphisms.

PO 033

Infertility and reproduction failure in autosomal translocations carriers

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Objective : Assessment of the incidence of autosomal translocation carriers in Tunisian subjects with reproduction failure and report of their treatment attempts results.

Design and Location : South of Tunisia during 5 years at the laboratory of Histology of the University of Medicine at SFAX.

Materials and Methods : Cytogenetic investigations were performed in 260 infertile men because of severe male infertility with low sperm count and in 47 couples because of recurrent miscarriages.

Results : Out of the 146 oligospermic and 114 azoospermic men, 35 had an abnormal karyotypes (13.5% : 21% in azoospermic and 7,5% in oligospermic men).

Chromosome aberrations observed were 24 sex chromosomal aberrations [comprising 46,XX (n=1), 47,XXY(n=20), 47,XYY(n=1) and 46,X,del(Yq) (n=2)] and 11 autosomal aberrations including 5 reciprocal translocations in oligospermic men [t(4;9)(p15.3;p21), t(11;22)(q24;q11),t(2;3)(p24;q26) t(16;22)(q13;q12) and t(11 ;21)(q13 ;p11)] and 6 robertsonian translocations [t(13;14) in 2 oligospermic and 2 azoospermic men and t(14;21) in an oligospermic and an azoospermic brothers]. The incidence of autosomal balanced structural abnormalities in oligospermic men was higher than in azoospermic men especially for reciprocal translocations (45,5% v/s 0%).

For robertsonian translocations carriers, a maternal meiosis transmission is recorded in two cases while for reciprocal translocations, a familial history is noted for 3 cases. In the other hand, chromosomal abnormalities were recorded twice out the 47 couples, in a female partner and in a normozoospermic male partner, who carried reciprocal translocations [46,XX,t(1;4)(q31;q26) and 46,XY,t (4;17)(p16;p12)]. None of theses translocations carriers come to conceive naturally or with assisted conception. In fact, fertilization failures in previous ICSI attempts (one to 5 attempts) were recorded for 6 couples.

Conclusion : Although ICSI provide a way of treating infertile men, it seems that translocations carriers need to be assisted with the most recent advances of reproductive technology which is the preimplantation diagnosis. In fact, only this technology may improve the implantation rates and protect couple from termination of pregnancy. Unfortunately, this practice is not yet available in our country.

PO 034

Preliminary study on the role of the human gene *izumo* in oocyte spermatozoa fusion failure

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Aim of study : Oocyte fertilization results from gametes fusion. Molecular complexes mediate this process. In ART, fertilization failure occurs in 10% of the attempts and fertilization is very often achieved by intra cytoplasmic sperm injection. This technique bypasses the physiological gamete membrane interaction with a risk of decreased embryo quality and genomic imprinting anomaly.

Izumo, first sperm membrane protein shown to be essential for fusion in mice, is hidden under plasma membrane and accessible only after the acrosome reaction. This novel immunoglobulin superfamily (IgSF), type I membrane protein with an extracellular immunoglobulin domain, was also detected in human and is encoded by a gene composed of 10 exons. We decided to look for mutations of the IZUMO gene in a group of infertile patients with a phenotype fitting to the features of the KO mouse model.

Design and Location : Retrospective and multicentric study.

Materials and Methods : Patients : 22 normozoospermic patients with past of intrauterine insemination failure followed by unexplained IVF fertilisation failure. 23 " controls " normozoospermic and fertile volunteers. 20 fertile men from general population (unknown semen parameters)

PCR : Amplification of the 9 coding exons of the gene (exon 1 is non-coding). Sequencing : mutation screening.

Results : No punctual mutation could be found in any of the 65 studied men. Three different polymorphisms were observed in exon 4 and intron 4 : c321C>T ; c397+17G>A ; c397+109G>A (neutral mutations without any predictable consequence on the protein) and one was observed in exon 10 (c998_999CG>TT) resulting in an alanine to valine substitution (pA333V). Only two combinations of these polymorphisms were observed (namely, C-G-G-CG and T-A-A-TT). There were no significant differences in the frequency of these genotypes in our 3 studied populations.

Conclusions : Two hypothesis :

1. IZUMO is implicated in gametes fusion in mouse but not in human where several proteins could have a redundant role in this function.

2. IZUMO is indeed implicated in gametes fusion in humans. Unfortunately, either we did not studied patients with the correct phenotype, or we missed the mutations (mutations outside the coding region or large size rearrangements). Therefore, protein expression needs now to be studied in infertile patients.

References : Inoue N., Ikawa M., Isotani A., Okabe M. : The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature, 2005, 10 ; 434(7030) : 234-8.

PO 035

Gamete aneuploidy rate and implantation failure

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Objective : Implantation failure (more than 10 embryos transfered without pregnancy) is a situation known to be associated to an increased risk of aneuploidy in embryos and considered as a PGD indication in many countries. Our aim was to evaluate the gamete aneuploidy incidence in this situation in France, a country where PGD is not allowed for this indication.

Materials : 3 couples groups were considered: group 1 (n=11) control couples with pregnancy obtained after IVF with fertile sperm and female infertility, group 2 (n=20) couples with pregnancy obtained after IVF for male or idiopathic infertility, group 3 (n=32) couples with implantation failure.

Methods : 1000 spermatozoa per patient were analysed by FISH (chromosome X, Y et 18 *(Abbott)* in the 3 groups. In group 3, 1st polar body (PB1) was also analysed by FISH with the *Polar Body*™ PGT multicolour *(Abbott)* kit, in the 22 couples who accepted the procedure during ICSI.

Results : Sperm aneuploidy rate was increased in group 2 (1,6% ; p<0,0005) and 3 (2,1% ; p<0,001) in comparison to group 1 (0,6%). In group 3, 2 patients presented a very high aneuploidy rate (>5%) in spermatozoa (6,5% et 25%). This

did not happen in group 1 and 2. 8/32 patients in group3 and 6/20 patients in group 2 exhibited a moderate but increased aneuploidy rate in sperm (between 2 and 5%). This did not happen in group 1.

PB1 aneuploidy rate was high (average:35,4%) in the 127 PB1 analysed in group 3. For 3 patients, this rate was very high, more than 2/3 abnormal oocytes, analysing only 5 chromosomes!

All together, 22% couples (5/22) had no particular chromosomal risk in gametes, 68% (15/22) presented an increased spermatic (> 2%) or oocyte (> 1/3) aneuploidy rate and 10% of couple (2/22) had both.

Conclusion : Those results confirm that implantation failure has a heterogeneous origin and that gamete chromosome abnormality rate is one of the major factors explaining it. Genetic counselling seems to be necessary after those analyses and those results may influence the next ART attempts, and future pregnancy chance. Indeed, preconceptionnal and when possible preimplantation diagnosis can be indicated for these couple (78% in our series) with increased gamete aneuploidy risk and implantation failure.

PO 036

Genetic counselling in azoospermia with primary ciliary dyskinesia

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Primary ciliary dyskinesia is a rare etiology of infertility in man. It is an autosomal recessive disease characterized by defective cilial ultrastructure in ciliated cells and affects about 1 in 20000 newborns. Regarded as a subgroup of primary ciliary dyskinesia, Kartagener's syndrome is characterized by the simultaneous presence of chronic bronchorrhea with bronchiectasis, chronic sinusitis and situs inversus. Infertility, occasionally described in males, is caused by ultrastructural defects of sperm tails with always total asthenozoospermia. We report a consanguineous family with two siblings, a male and a female, who have a typical Kartagener's syndrome and another dead brother who seems to be affected. The diagnosis was established on the basis of clinical grounds and familial history of a 33-year old man who was referred for cytogenetic exploration because of 11 months history of male infertility with azoospermia in semen analysis.

Clinical history and physical examination revealed presence of situs inversus totalis, chronic bronchorrhea and bronchiectasis, chronic nasal symptoms, sinusitis and infertility. Serum FSH, LH and testosterone were normal and the patient had a normal 46,XY karyotype. The patient's sister was also affected and had similar features with the same respiratory symptoms and situs inversus totalis. After some difficulties to conceive, she gave birth to a healthy female child and a male stillborn with multiple congenital abnormalities. Their third sister had also history of foetal congenital abnormalities. Diagnosis of Kartagener's syndrome was confirmed by electron microscopy study of ciliary structure. Epithelial cells were obtained from nasal and bronchic biopsy specimens. Ciliary ultrastructure showed qualitative defects in dynein arms with absence of the outer dynein arm and an abnormal additional microtubule at the central doublet probably secondary to radial spokes abnormalities.

Genetic diagnosis consisting of mutation research in DNAI1 gene at 9p13-p21 (20 exons), DNAH5 gene at 5p15-p14 (79 exons) and DNAH11 gene at 7q21 (82 exons) may be helpful for familial genetic counselling but remains hard because of the extent of these genes and heterogeneity of the disease.

The infertile patient, who sought an ICSI treatment, was informed about possible rates of testicular sperm retrieval after TESA (Testicular sperm aspiration) and all possible genetic risks through a genetic counselling.

PO 037

Transcriptional study of apoptosis during the first wave of spermatogenesis in gonadotropin deficient mice

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Objectives : The objective of this study is to investigate the transcriptional events involved in the infertility phenotypes seen in luteinizing hormone receptor Knock-out (LuR-KO) and Hypogonadal (hpg) male mice. The LuR-KO lacks the

receptor for LH causing the failure of Leydig cells to secrete androgen. Therefore spermatogenesis arrests at the round spermatid stage and leads to male infertility. Hpg lacks GnRH preventing the pituitary from secreting either LH or FSH. These hpg mice are also infertile with spermatogenesis arrest at the pachytene spermatocyte stage. Here we concentrate on apoptosis pathways in the testis during the early stages of spermatogenesis.

Design : Microarray analysis was used to identify biological processes that are differentially expressed during the premeiotic and meiotic stages of the first wave in the two mouse models compared to the wild type. Apoptosis was found to be one of the mechanisms highlighted by this study. TUNEL assays were performed to confirm the occurrence of apoptosis. Transcriptional profiles of some genes were confirmed by QRT-PCR.

Materials and Methods : Microarray experiments were performed comparing the models to an age matched control on the MmcDNAv1 library. Each experiment included 4 technical replicates and 2 biological replicates. Technical reproducibility was assessed using a windowing procedure described by C.Tseng. The Z score method described by J. Quackenbush was used to identify differentially expressed genes. Source was used to categorise genes into different GO groups (gene ontology). GO data was normalised taking into account the library composition and the number of genes differentially expressed in each experiment. TUNEL assays (Apoptag kit, Chemicon) and immunohistochemistry were performed on paraffin sections. Apoptotic figures were also identified on H&E sections.

Results : Differentially expressed genes for each model at each time point are highlighted in figures 1 and 2. The number of genes affected by the mutation increases in the last 3 time points in LuR-KO, possibly due to increasing differences in cell composition.

Figure 1 : MA plots for LuRKO at day3, day 8, day 13 and day 19. The number of genes differentially expressed is indicated for each time point, and they are labelled in red. For hpg, the number of differentially expressed genes increases at each time point and is larger than in LuR-KO. The lack of both gonadotropins has a strong effect on gene expression.

Figure 2 : MA plots for hpg at day3, day 8 and day 13. The number of genes differentially expressed is indicated for each time point, and they are labelled in red.

GO data indicates that, at the transcriptional level, apoptosis genes are over represented in the model at all time points except at day19 in LuR-KO. Most apoptosis related transcriptional events occur at day 8 in both models. In hpg at day 8, there is a bias towards apoptosis related genes in the list of differentially expressed genes (almost 2.5 times the expected amount). Figure 4 presents a selection of genes whose expression is altered in the models. Figure 5 indicates localisation of the transcripts during the WT first wave of spermatogenesis.

Figure 3 : Proportion (% compared to WT) of genes related to apoptosis that are differentially expressed at the different time points. This data is normalised to the overall composition of the library.

Figure 4 : Expression level of selected genes involved in apoptotic pathways in the model compared to the age matched WT. Only genes with a fold change >1.5 are shown. The anti-apoptotic genes are shown in green, the pro-apoptotic in orange and the regulators of apoptosis in grey. Eg: Aven is 1.5 fold down regulated in LuR-KO at day 3 compared to the age matched control.

Transcript	Localisation
Bcl2l11/Bcl-Rambo	PGC (Tanaka et al 2002)
Faf1	Spermatogonia, Spermatocytes , Spermatids, Somatic cells (Ryu et al, 1999)
Api5	Spermatogonia, Spermatocytes 1 , Somatic cells (Clemente et al, in press)
Btg2	Spermatogonia, Spermatocytes 1 , Somatic cells (Clemente et al, in press)
Bcl2l11/Bim	Spermatogonia, Spermatocytes , Spermatids, Somatic cells (Meehan et al, 2001)
Bcap29	Spermatocytes, Spermatids (Clemente et al, in press)
Dap	Spermatogonia, Spermatocytes , Spermatids (Clemente et al, in press)
Btg2	Spermatogonia, Spermatocytes 1 , Somatic cells (Clemente et al, in press)
Aven	Spermatogonia, Spermatocytes , Spermatids (Ina et al,2003)
Diablo	Spermatocytes (El Chami et al, 2005)
Cflaf/Flip	Spermatocytes (Giampretri et al,2003)
Fasl	Spermatocytes, Spermatids (D'Alessio et al, 2001)
Daxx	Spermatocytes, Spermatids,Somatic cells (Lopez et al 2002)

Figure 5 : Known localisation in wild type testis of the selected transcripts involved in apoptosis.

Figure 6 : TUNEL assays. Histology presents TUNEL assay results. Apoptotic cells are labelled in brown and are mainly of later stages of spermatogenesis. The histogram presents the average number of apoptotic cells per tubule, p value <0.1 is indicated with a star (data is not yet available for hpg adult).

Conclusions : As confirmed by TUNEL assays (Figure 6), apoptosis is involved in eliminating the germ cells that stop differentiating in hpg and LuR-KO testis. Immunohistochemistery results for active Caspase 3 and Cytochrome-c should be available for the conference. This will allow a better understanding of the apoptotic pathways induced during the first wave of spermatogenesis and how they might contribute to the infertility phenotype in the two models.

Support : Our work is funded by the Department of Pathology at Cambridge University.

PO 038

The effects of Human Chorionic Gonadotropin on germ cells maturation and testosterone secretion in mouse testis

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Objectives : Testis function including sperm production and androgen secretion is controlled by hypophyseal gonadotrophins. Androgen production is induced by luteinizing hormone (LH) and promotes germ cell proliferation and maturation. LH has more than 90% similarity with Human Chorionic Gonadotropin (hCG) in structure and function, accordingly hCG is used in research and clinic in spite of LH presence. The aim of this study is to evaluate the effects of different dosages of hCG on germ cell maturation and testosterone secretion in immature testis of neonate mouse.

Design : The present study was designed to examine testosterone secretion and germ cell proliferation in mouse. Moreover, the long-term effects of this hormone have been evaluated on the germ cell proliferation. Because the effects of hCG could be dose related, varying doses of hCG were used. Simultaneously, the effects of hCG injection on androgen status of the animals were evaluated.

Materials and Methods : 24 mice (C57BL/6) aged 15 days old divided into 4 equal groups that group 1 served as control.

Table1 : Serum testosterone levels on day 28.

Group	Testosterone level (nmol/l)	P value
1	0.2± 0.61	
2	1.18± 1.64	NS
3	1.3± 2.62	NS
4	3.56± 4.75	0.05 <

NS = no significant difference. Data presented as the mean ± SD.
Group 1 is the control group.

Table 2 : Serum testosterone levels on day 65.

Group	Testosterone level (nmol/l)	P value
1	7.74 ±2.78	
2	0.81 ±1.00	NS
3	0.63 ±1.07	NS
4	0.57 ±0.73	NS

NS = no significant difference. Data presented as the mean± SD.

Table 3 : Percentage of haploid cell population of testis on day 65.

Group	Haploid cells (%)	P value
1	2.38± 70.67	
2	4.70± 68.46	NS
3	13.12± 55.67	0.05 <
4	12.32± 36.69	0.05 <

NS = no significant difference in haploid cell population percentage.
Data presented as the mean ± SD

The groups 2, 3, and 4 were exposed to 5, 10 and 50 IU hCG respectively that was injected on days 15 and 25 of the mouse life. The level of serum testosterone was determined on days 28 and 65 of the mouse life. The different testicular cell populations were determined according to DNA contents by Flow Cytometric Analysis. The data thus obtained were analyzed by Cell Quest software. Statistical analysis was done using SPSS software program. The Kruskal-Wallis test was applied for comparison of all groups and Mann-Whitney test was applied for comparison of any group with the control group and P values less than 0.05 were considered statistically significant.

Results : Day 28 testosterone in case groups were shown to have increased, as compared to the control group by increase in hCG dose, with the highest values in group 4 as shown in Table 1. However, only the difference between group 4 (highest hCG dose) and the control group was found to be significant. In contrast to day 28, different results were found for day 65. In this regard, the testosterone levels were declined with increasing dose of hCG in different groups in comparison to the control group. Accordingly the group 4 had the lowest level of testosterone (Table 2). There was also no statistically significant difference detected between the case groups and the control group. Additionally, DNA Flow Cytometric analysis revealed that the germ cell numbers had remarkably declined in case groups in comparison with the control group on day 65 and this reduction was statistically significant for groups 3 and 4 (Table 3).

Conclusions : The present study used a mouse model and demonstrated that hCG, administrated at levels comparable to clinical dosages, adversely affects the testicular germ cell population and androgen production. With maturation, there is an increase in haploid cell proportion with a simultaneous

decrease in the other cells. Thus, the significantly reduced haploid cell population seen postpubertally in group 4 in the present study is consistent with these reports and implies impaired germ cell maturation. In the present study, only a profound reduction in testosterone levels, as seen in group 4, was associated with a reduced haploid cell population. This might imply that mild to moderate androgen withdrawal may be safely tolerated by the germ cells. It may be that Leydig cells are also sensitive to androgen withdrawal; however, additional studies are necessary in this regard.

Support : This study was supported by Avesina Research Center.

PO 039

Transition protein expression in spermatids during the first spermatogenic wave in golden hamster

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Objective : Spermatogenesis is a sequence of events which starts in germinal cells (spermatogonia) and ends in mature sperm cells (Spermatozoa). The process consists of spermatogonial proliferation, meiosis and spermiogenesis (spermatid differentiation). In spermiogenesis, the round spermatids, the product of meiosis, are the first haploid cells. They undergo biological, morphological and cytological differentiation to produce mature spermatozoa. Among other changes histones in the chromatin are presumed to be replaced, first by transition proteins (TPs) and then by protamines. The objective of the present project was to determine the age of hamsters and step of spermiogen-esis where TPs are first expressed.

Design : Male hamsters undergoing the first spermatogenic wave, from 26-42 day postpartum (dpp), were sacrificed by CO2 asphyxiation ; testes were surgically excised and single cell suspensions were prepared for flow cytometric analysis. In addition, a portion of each testis was fixed in Bouin solution, embedded in paraffin and prepared for staining on slides for confocal laser scanning microscopy (CLSM).

Materials and Methods : The single cell suspensions were analysed by flow cytometer after staining with Propidium Iodide (PI). The slides were incubated in citrate buffer (0.1 M) at 900C for 10 minutes. Antibodies against transition proteins TP1 and TP2 (generously provided by Dr. W. S. Kistler) were added and incubated overnight, washed (3 times in Triton x-100 0.1%) and secondary antibody (goat anti rabbit IgG conjugated with FITC) was added for fluorescence microscopy. In order to correlate between dpp where TP were first expressed and the step of spermiogenesis, fixed tubuli from each dpp were stained with PI and analyzed by CLSM. On each dpp we determine the most advanced spermiogenic step by focusing on the spermatocyte and spermatid layers.

Results : Using fluorescence microscopy TP2 appeared at 27dpp (Step 6), and peaked at 34 dpp during maturation of spermatids during spermiogenesis. TP1 appeared later (at 28dpp, step 8) and peaked at 35dpp. Using flow cytometry we could demonstrate that the chromatin condensation process started before the appearance of TPs.

Conclusion : Confocal microscopy demonstrated that TP2 was first expressed in step 6 and TP1 in step 8 during spermiogenesis in the hamster. Chromatin condensation in the hamster started before the expression of TPs.

Support : In part, by a grant of Israel Ministry of Health Chief Scientist Office.

PO 040

Analysis of gonosomic aneuploidy in men with severe teratozoospermia

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Objective : The aim of this study is to evaluate the incidence of spermatic aneuploidies in men with severe teratozoospermia and to determine an eventual relation between aneuploidies and a specific morphology of spermatozoa.

Material and Methods : Fluorescent in situ hybridization (FISH) with direct label fluorescence DNA probes specific for chromosome X, Y and 8, was performed on spermatozoa sampled from 30 patients with severe teratozoospermia (abnormal forms > 80%). Results were compared with those of spermatozoa sampled from 12 healthy men with normal semen profiles.

Results : A minimum of 10000 spermatozoa was analysed per chromosome probe. The mean frequency of teratozoospermia in patients was 91 ± 6.99%. There was a statistically significantly increased frequency of XY, XX and YY disomies in the patients with severe teratozoospermia compared with the normal donors (1.42 vs 0.31%, 1.13 vs 0.19% and 1.11 vs 0.24%, respectively, $p<0.001$ in all comparisons). The rate of total diploidy was significantly increased in the patients compared with normal donors. The difference was statistically significant (1.46 vs 0.16%, $p<0.001$). The sex-chromosomal anomalies due to the meiosis I (XY) are less important than the anomalies due to the meiosis II (XX or XY).

There was a correlation between macrocephalic spermatozoa and diploidy ($r = 0.37$, $p<0.05$) and a correlation between macrocephalic spermatozoa and nullisomy XY ($r = 0.48$, $p<0.05$).

Conclusion : These data add further evidence that patients with severe teratozoospermia have an increased sperm aneuploidy rate and that this is particularly high in macrocephalic spermatozoa.

PO 041

Expression of Synaptonemal Complex Protein 3 (SYCP3) mRNA in Testis : A Possible Molecular Marker for Spermatogenesis in Patients with Azoospermia

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Objective : To investigate the expression of the Synaptonemal Complex Protein 3 (SYCP3) gene as a molecular marker for spermatogenesis in men with non-obstructive azoospermia.

Design : Cross-sectional case study.

Materials and Methods : We investigated testicular samples of 110 men with non-obstructive azoospermia in Avesina Infertility Clinic, Tehran, Iran during 2005-6. Semi-quantitative nested RT-PCR was performed in order to determine the expression of SYCP3. We also evaluated the expression level of gene during spermatogenesis using the histopathological scoring of all samples.

Results : Testicular expression of SYCP3 mRNA was observed in 67/110 (60.9%) patients. The expression level correlated with the level of spermatogenesis ($p<0.0001$). It was expressed in patients with hypospermatogenesis and maturation arrest, while expression was not observed in spermatogonial arrest, Sertoli cell– only syndrome and testicular atrophy. Sensitivity, specificity, positive and negative predictive values of negative SYCP3 expression for detection of patients with Sertoli cell-only syndrome were 88.5%, 92.3%, 86.1%, 93.7%, respectively.

Conclusion : our findings indicate that SYCP3 is expressed in human testis and is restricted to germ cells. The gene expression in a specific level of spermatogenesis can help histopathological findings in prediction of level of spermatogenesis. Therefore, SYCP3 is suggested as a possible molecular marker for spermatogenesis in men with non-obstructive azoospermia.

Support : This research was supported by Molecular Medicine Network, Ministry of Health & Medical Education, Iran.

PO 042

Longitudinal study of inhibin B values during childhood and puberty in a boy with Klinefelter's syndrome

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Objective : Klinefelter's Syndrome (KS) is characterised by degenerative changes in the seminiferous epithelium, resulting in fibrosis of the seminiferous tubules and Leydig cell hyperplasia. This degenerative process leads to the typical small, firm testes seen with this syndrome. It mainly affects germ and Sertoli cells, although their numbers range from

normal to subnormal during infancy (Muller et al., 1995). In normal boys Inhibin B (InhB) levels progressively increase throughout puberty, until they reach normal adult values as a marker of mature spermatogenesis (Radicioni et al., 2005).

Design : Case presentation, clinical and laboratory evaluation.

Clinical and Laboratory Evaluation : We studied a KS boy (karyotype 47,XXY, early diagnosis by amniocentesis) from the age of 4.5 to the end of puberty. At the first visit, his height was cm 107.5 and weight kg 18.200, with mean testicular volume (MTV) 1.0 ml (Prader orchidometer) and parenchymatous consistency. Subsequent visits documented a regular height growth curve and hormone levels and testicular volumes as reported in the table. Spontaneous puberty began aged 12.5 yrs with regular progression to stage G5 (Tanner, 1962).

Conclusions : These data demonstrate childhood and early puberty InhB levels similar to normal values. Mid-puberty saw a rapid drop in gonadal hormone. This suggests – in our case at least – that Sertoli cell function is normal up to stage G3. Following this, in normal subjects spermatogenesis activity begins and InhB originates from the communication between Sertoli and spermatogenesis cells, whereas in our patient InhB values underwent a rapid decrease.

FSH in childhood and early puberty was also found to be within the normal range for the age, and began increasing on entering mid-puberty. Testicular volume increased up to the beginning of spontaneous puberty and underwent a further small growth the following year, at which point penis morphology and pubic hair growth showed a normal progression.

PO 043

Effect of cowhage (*mucuna pureins*) on reproduction in male albino rats

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Objective : To test alcoholic seed extract of cowhage (Mucuna pruriens) for aphrodisiac and spermatogenic potential in male albino rats.

Design : Albino rats weighing around 180 to 200 gms were classified into various groups, group I (G1) received saline and group II (G2), group III (G3) and group IV (G4) received 150, 200 and 250 mg/kg of body weight of extract (seed) respectively. Animals received daily oral feeding of either extract or saline for 50 days.

Materials and Methods : Following investigations were done on 15th, 30th and 50th days of survival 1.Mating behavior : Computer analysis of the video recorded mating behavior using responsive female rats. Parameters analyzed were mount latency, intromission latency, ejaculation latency, post-ejaculatory interval, mounting and intromission frequency. By 60th day, animals were sacrificed by over dose of anesthesia. 2. Sperm analysis : Epididymal sperm were collected and following parameters were analyzed; sperm concentration, viability and motility. 3. DNA/ Chromatin integrity test : Sperm smear was stained with acridine orange (AO), aniline blue (AB) and methyline blue (MB) to study DNA integrity. 4. Biochemical and Hormonal analysis : Blood was collected periodically were used for these analyses. 5. Anti-oxidant, histological and histomorphometrical study : Testis and epididymis were carefully collected and weighed individually; tissues were processed for anti-oxidant (superoxide dismutase, reduced glutathione, glutathione reductase, glutathione transferase, glutathione peroxidase and vitamin C and E) and Hematoxylin and Eosin stained sections were used histological and histomorphometric analysis.

Results : Biochemical values indicate mild toxic effect in G4. There was an increase in sperm concentration, in G2, G3, and G4, when compared with G1, peak was in G3. However, number of sperm cells with chromatin integrity was slightly reduced in drug administrated groups, than control. Similarly, mating behavior scoring indicates an increase in ejaculation time and number of intromission, which was more pronounced in G3, than G2 and G4. Testosterone level was increased by 15th and 30th days in G2, G3 and G4. However; there was no progressive increase with course of dose, but maintained at a higher level than normal. Anti-oxidant levels were near normal in G3, whereas slight increase was observed in others (G2, G3 and G4). Increase in testicular and epididymal weight was seen in experimental groups. Histological and histomorphometric study revealed increased spermatogenic activity in G2, G3 and G4.

Conclusion : Increase in ejaculation time, number of mounting and sperm concentration signify that seed of this plant is having the potential to increase sexual activity and sperm production. Effects seem to be dose dependant as G3 exhibit excellent result. However, reason for more number less, sperm with DNA/chromatin integrity, might be due to increased (could be premature) spermiation or /and less time given for sperm to mature in the ducts before being ejaculated. Increase in testosterone level signifies the androgenic property of the extract. Testicular and epididymal weight increase supported by histological analysis clearly demonstrate that, the cowhage having the potential to increase sperm production, as well as producing aphrodisiac effect in male rats. However, a long-term study is warranted to analyze its beneficial and toxic side effects of this extract, before testing it on specific fertility problems.

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PO 044

Regional variations in semen quality in France

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Several studies have suggested that the semen concentration of fertile men has declined in recent decades in many industrialised countries. In France, a retrospective study of sperm donors from 1973 to 1992 found a decrease in sperm concentration in a northern region (Paris) over the study period. Nevertheless, no sperm concentration decrease was observed in south-western France (Toulouse) during the same period (1977-1992). For the authors, these findings may suggest a regional difference in semen quality which could be due to different environmental exposures between the two French regions. Nevertheless, these conclusions must be taken with caution due to potential bias linked to the long inclusion period (starting in 1973 and ending in 1992) with possible modifications in sperm analysis methods during this time.

Objective : In order to re-analyse geographical variations in semen concentration between north and south-western France, we conducted a prospective study (REPRHOM) of sperm characteristics, using standardised methodology and including laboratory quality control.

Design, Materials and Methods : From 2002 to 2003, male partners of pregnant women were recruited in maternity departments in Paris and Toulouse, using the same standardised recruitment protocol. A semen sample was collected from each volunteer. Quality control showed no significant inter-laboratory variability in sperm concentration assessment.

Results : During the study period, sperm characteristics were examined in 129 subjects living in a northern region of France (Paris area) and 79 in a south-western area (Toulouse). No difference was observed in sperm concentration, mobility or other characteristics between men living in Paris and in Toulouse.

Conclusions : Our results are in contradiction with those of an earlier study where sperm quality was found to be higher in men living in Paris compared with those living in Toulouse, between 1973 and 1992. Although the authors suggest that the environment may play a role in such regional variation, recruitment and measurement bias cannot totally be excluded (recruitment began 30 years ago and continued for 20 years). Moreover, even if this environmental hypothesis could be considered to have an impact in the 1970s, we believe that pollutant exposure has drastically changed 30 years later, especially in the south-west of France where intensive prevention has limited environmental pollution, especially for pesticide exposure. In conclusion, our comparative study did not confirm regional variations in sperm characteristics between north and south-western France in the early 2000s.

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PO 045

Impact of occupational and environmental exposures on semen parameters : evaluation of an investigation methodology.

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Objective : To study relations between occupational and environmental exposures and semen parameters.

Design and setting : Retrospective study between novembre 2004 and november 2005, La Conception University Hospital, Marseille, France.

Materials and Methods : Population: 340 volunteer patients (aged 18 to 55) consulting for infertility evaluation in Reproductive Medicine Laboratory. Methods: association of Reproductive Medicine Laboratory, Occupational Medicine Unit and Genetic Toxicology Laboratory. Patients exposures were assessed by an occupational physician before semen analysis, using a standardized questionnaire. Semen parameters were assessed according WHO criteria. Statistical analysis : Chi-squared test, ANOVA means comparisons and multivariable logistic regression model.

Results : Population : 80% of patients showed abnormal semen parameters, 20% showed normal semen parameters (considered as control patients). Main occupational sectors were: office workers (15% of patients), professional drivers (14%) and building trade workers (11%). Statistical analysis: no occupational sector was over-represented in patients with abnormal semen parameters, but this population was significantly more exposed than control patients to: solvents, colouring, gaz and fumes, cement, polycyclic aromatic hydrocarbons, heat and cannabis ($p<0.05$). Analysis by logistic regression showed that the risk for abnormal semen parameters was associated with exposure to cement (OR = 5.2 ; p = 0.022), cannabis (OR = 4.0 ; p = 0.047) and environmental pollution (OR = 3.8 ; p = 0.052).

Conclusions : The findings indicated an association between impaired semen parameters and exposure to some environmental or occupational toxic agents. This approach could be systematically used during infertility evalutation, in order to assess reproductive toxicant exposure of patients. The perspectives are to complete the investigation by the use of biomarkers of exposure in semen.

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PO 046

Estrogens regulate epididymal contractility through RhoA/Rho-kinase signalling

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Objective : Epididymis is a sex steroid-sensitive duct provided with spontaneous motility, allowing sperm transport. We previously demonstrated that human epididymis expresses an high abundance of mRNA for ER-alpha and ER-beta. We also demonstrated that in epididymis estrogens up-regulate either OT responsiveness, acting at the receptor level, and responsiveness to endothelin-1 (ET-1), another well known stimulator of epididymal motility. However, we did not find any significant change either at gene or protein level in ET-1 and its cognate receptors. Hence, other molecular effectors should mediate the increased sensitivity to ET-1. In particular we hypothesized that estrogens up-regulate some contractile effectors, such as RhoA/Rho-kinase pathway, downstream to the ET-1 receptors.

Design and Methods : To investigate the effect of changing endocrine milieu on RhoA/Rho-kinase pathway, we induced hypogonadism (hypo) in rabbits with a single administration of 2.9 mg/Kg of a long-acting GnRH analog, triptorelin, and we replaced hypo rabbits with different sex steroids (Testosterone, T 30 mg/Kg weekly or estradiol valerate, E2, 3.3mg/Kg weekly).

Results : After 8 weeks from GnRH analog administration, T plasma levels were decreased and the relaxant effect of the Rho-kinase inhibitor,Y27632 on ET-1 pre-contracted epididymal strips, was significantly decreased. T administration restored T plasma levels, but not Y27632 sensitivity in the epididymal strips. E2 not only completely restored Y27632 responsiveness but even amplified it, as indicating that the RhoA/Rho-kinase calcium sensitizing pathway is up-regulated by E2. Accordingly, RT-PCR studies indicate that Rho kinase gene was strongly induced by E2 but not by T. To verify whether endogenous estradiol is involved in the regulation of Y27632 responsiveness, we treated intact rabbits with an aromatase inhibitor, letrozole (1.25 mg/day) for 21 days. Blocking aromatase activity abolished Y27632 responsiveness in rabbit epididymis.

Conclusions : Our results support the hypothesis that epididymis is a male target for E2, which regulates its motility tuning up contractile hormones and local peptides responsiveness by increasing RhoA/Rho-kinase signalling and therefore calcium sensitivity.

PO 047

An in vitro model for epididymal sperm maturation

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Objective : The fertilization potential of spermatozoa is critically dependent on maturation in the epididymis. One of the key features is progressive motility, which is gradually acquired during the epididymal transit. Cellular and molecular mechanisms underlying epididymal sperm maturation, however, have not yet been elucidated in detail. In order to investigate the interaction between epididymal epithelium and spermatozoa during this process, we developed a co-culture system of epididymal epithelium and spermatozoa in a defined, serum-free culture system.

Material and Methods : Primary cell cultures of epithelial cells obtained from caput, corpus and cauda epididymis were grown in culture medium (RPMI 1640) supplemented with 5% fetal bovine serum until confluence. On the day of confluence (usually day 6 after preparation), cell cultures were washed twice with serum-free medium. The same medium was used for further cultivation. Spermatozoa freshly obtained from bovine caput epididymis were added to the epithelial cell cultures at a concentration of 10 x 10^6 /ml. As a negative control, caput spermatozoa were incubated with fresh serum-free medium alone. Motility was measured with a CASA system (Microptic, Spain) every 24 hours until 72 hours of co-culture.

Results : Caput spermatozoa showed a low rate (7%) of global motility immediately after preparation. Global motility increased in both, negative control (20%) and co-cultures with epithelial cells of all three epididymal regions (50%) after 24 hours incubation time. However, changes in motility were significantly lower in the control group. Global as well as progressive motility in the co-culture setting was maintained at high values until termination of the experiment after 72 hours. In contrast, global motility of the negative control was largely reduced after 24 hours. Furthermore, spermatozoa cultivated in unconditioned medium showed a significant loss of vitality associated with a high level of disintegration, i.e. between head and flagellum.

Conclusions : Our data demonstrate that a co-culture system of epithelial cells from bovine caput, corpus and cauda epididymis with bovine caput spermatozoa is able to trigger the development of global and progressive motility. In contrast to unconditioned serum-free medium, the epididymal environment maintains sperm vitality and motility for at least 72 hours supporting the key role of epididymal cell-cell interactions for sperm maturation.

PO 048

Sperm phagocytosis by mononuclear cells in the ejaculate : a marker of epididimydis pathology ? A quantitative ultrastructural analysis

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Objective : Experimental epididymidis obstruction is associated with appearance in the epididymal lumen of macrophages undergoing sperm phagocytosis. This is the result of epididymal barrier disruption and exposure of sperm to the immunosystem. Macrophages undergoing phagocytosis of sperm and bacteria (Ma-Phago) are observed by ultrastructural analysis of ejaculate in infertile men.

Design : We analysed in a large number of ejaculates evaluated by quantitative ultrastructural analysis with transmission electron microscope (TEM) the relationship between the presence of Ma-Phago as a marker of sperm exposure to the immunosystem, and seminal parameters.

Materials and Methods : 430 ejaculates with a leukocytospermia < $1x10^6$/ml and a negative MAR test, were analysed by routine semen analysis and by TEM. Specimens were grouped on the basis of the presence by TEM of Ma-Phago. The quantitative ultrastructural analysis included the fraction of heads with normal chromatin and acrosome organization, the fraction of degenerating sperm with fragmented membranes, the fraction of sperm with amorphous heads (altered shape associated with altered chromatin condensation and a disorganized acrosome).

Results : 26.7% of samples showed Ma-Phago. Compared with the others, these showed a decreased number of sperm total count ($p<0.0001$) and a reduced sperm forward motility ($p<0.04$) but no difference for sperm morphology. The quantitative TEM analysis showed that group with Ma-Phago showed a significative incresed fraction of degenerating sperm

(p=0.0002), and of amorphous heads (p=0.019). The fraction of degenerating sperm was correlated negatively with sperm forward motility (r=0.35, p<0.0001) and sperm total count (r=0.30; p<0.0001). A multivariate logistic analysis showed that a low number of total ejaculated sperm and an increased number of degenerating sperm indipendently predicted the presence of Ma-Phago (OR 1.72; CI 1.10 to 2.28 and OR 1.85 CI 1.19 to 2.88 respectively).

Conclusion : The presence of macrophages undergoing phagocytosis of sperm and bacteria is frequently observed in ejaculates of subfertile men and is associated to a decreased number of sperm count but normal morphology, suggesting it may be a marker of an inflammatory sub-obstruction at epididymal level. **Supported** by MURST, PRIN 2005

PO 049

Correlation of apoptosis and aneuploidy in spermatozoa of oligoasthenozoospermic patients

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Objective : To evaluate the cytogenetic constitution of spermatozoa from twenty oligoasthenozoospermic (OA) patients by analyzing the rates of aneuploidy for the chromosomes 13, 18, 21, X and Y and to determine if any correlation exists between the DNA fragmentation and the incidence of sperm chromosome aneuploidy.

Design : Basic research laboratory experiment.

Twenty patients (aged 24-50, mean age : 37.3±7) were referred to the department of Assisted Reproduction with the view of participating in an ICSI program, after experiencing at least two years of infertility. Semen analysis was carried out, based on the WHO 1999 guidelines and a severe male factor condition was the main cause of infertility due to oligoasthenozoospermia (n=20).

Materials and Methods : Dual and triple colour FISH (Fluorescence in situ hybridization) was applied, according to standard manufacturer procedures (Vysis, Inc.). The probes 13, 18, 21, X and Y were used and the stained samples were observed using an Axiolab Zeiss microscope. A total of 20.000 spermatozoa were counted.

Nick end labelling of DNA breaks in sperm nuclei have been performed with a mixture of biotinylated dUTP (Roche, stock 50 nmol) and TdT (Amersham, stock 9 units/µl) in TdT buffer. After the end of the reaction, the slides were incubated with Texas Red Streptavidin (1mg/ml), which revealed the apoptotic nuclei, and the non-apoptotic nuclei were counterstained with DAPI (1mg/ml) (Vector Laboratories). At least 500 spermatozoa per sample were evaluated using a fluorescent microscope.

Statistical analysis was performed with SPSS-PC V11.0 Software. As all continuous variables were symmetrically distributed (as shown by analysis of variance using the Shapiro-Wilk test) the parametric Pearson's r correlation test was used to examine the relationship between sperm aneuploidy and TUNEL reactive spermatozoa. A p value of <0.05 was considered statistically significant.

Results : The mean percent aneuploidy for all five chromosomes was 1.35, while the percentage of TUNEL positive spermatozoa was 45.75. No statistically significant correlation (r=-0.407, p=0.075) was observed between chromosome aneuploidy (five chromosomes) and TUNEL reactive spermatozoa.

Conclusions : The data obtained from the 20 oligoasthenozoospermic patients showed that the total sperm chromosome aneuploidy for five chromosomes (X, Y, 13, 18 and 21) did not correlate with sperm deoxyribonucleic acid fragmentation (evidenced by TUNEL assay).

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PO 050

Ultrastructural nuclear defects and increased chromosome aneuploidies in spermatozoa with elongated heads

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Objective : Elongated spermatozoa are characterized by a head length higher than 5µm. Cellular and molecular mechanisms leading to elongated sperm heads are not known. An increased nuclear volume has been reported to be associated with sperm head abnormal elongation. The incidence of sex chromosome abnormalities is increased

significantly in morphologically abnormal spermatozoa (macronuclear spermatozoa). Whether anomalies of the chromosomal content are responsible for the increased nuclear volume of elongated spermatozoa is questioned.

Design : The nuclear status of elongated spermatozoa was evaluated by ultrastructural and cytogenetic analyses.

Setting : Teaching hospital.

Materials and Methods : Fourteen men with at least 30% of spermatozoa with an elongated nucleus. Five fertile men were used as controls. Medical history, family infertility and personal habit were recorded. Semen parameters, semen biochemical markers, karyotypes and Y chromosome microdeletions were assessed in all patients. Sperm morphology was analysed by a quantitative ultrastructural analysis. Sperm chromosomal content was assessed by three colour fluorescence in situ hybridisation (chromosomes X, Y, 18). Sperm aneuploidy rates were compared between patients and controls with a Mann-Whitney statistical test. Differences were significant when $P<0.05$.

Results : Semen parameters (median) of the patient group as compared to the control group were ranged as follows: total sperm count (67.1 Mo ± 10.6 vs 219 Mo ± 47), percentage of progressive sperm cells (25% ± 10 vs 25% ± 15), sperm viability (66% ± 18 vs 72% ± 22) and normal spermatozoa (3.5% ± 3.5 vs 69% ± 5). The patients presented a polymorphic teratozoospermia, where a majority of spermatozoa display more than one type of abnormality. Elongated sperm head rates were ranged from 30% to 75% (median : 48.5%) in the patient group versus 0% to 2% in the control group. No anomalies of sperm biochemical markers were found in either group. All the men showed a normal karyotype (46,XY) and an absence of Y chromosome microdeletion. Quantitative ultrastructural analysis revealed the presence of nuclei of various sizes and shapes, including a mean of 13% of spermatozoa with an increased nuclear volume and a mean of 10% of binucleated sperm cells. Moreover, the chromatin was undercondensed in 30% of the spermatozoa. Aneuploidy rates of gonosomes and chromosome 18 were significantly higher in patients with elongated sperm heads as compared to the values of control sperms (1.8 and 4.3 fold increase, $p=0.02$ and $p=0.006$, respectively).

Conclusions : This study demonstrates for the first time that the chromatin compaction and the numerical chromosome content can be altered in spermatozoa with an elongated head and that elongated sperm nuclei could arise from meiotic non-disjunctions.

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Characterization of M540 bodies present in human semen

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In semen of subfertile subjects, membrane-surrounded round bodies (termed as M540 bodies) are present. M540 bodies stain promptly with merocyanine 540 (M540), a probe revealing somatic apoptosis. M540 bodies appear virtually devoid of chromatin as nuclear dyes fail to stain them. Further, M540 bodies have heterogeneous size and density and result ubiquitinated. Finally, their amount correlates with poor quality semen parameters.

In this study we challenged the hypothesis that M540 bodies are apoptotic bodies, derived from testis apoptosis. By flow cytometry, we investigated whether M540 bodies exhibited apoptotic key markers, by excluding from the analysis semen nucleated cells.

We found that M540 bodies express all the apoptotic markers investigated : caspases activity, Fas receptor, p53 and TUNEL positivity (percentages of positive M540 bodies were respectively: 68.1±20.7% ; n=7 ; 13.7±10%, n=6 ; 42.2±13.5%, n=4 and 22.2±18.7, n=8, mean±SD). The latter parameter increased after treatment with exogenous nuclease, suggesting that M540 bodies do contain fragmented DNA.

In this study we show that M540 bodies resemble closely apoptotic bodies as they express key apoptotic markers. Occurring of DNA fragmentation was surprising to us. On the other hand, if M540 bodies are apoptotic bodies, their DNA content is expected to be very low but intensively fragmented. In agreement with this, our data show that DNA bound to M540 bodies is detectable by TUNEL but not by nuclear staining. In M540 bodies, caspase activity occurs at a greater extent than Fas receptor, p53 and DNA fragmentation. The higher positivity observed for caspases with respect to Fas, p53 and DNA fragmentation could be explained by the different localization of caspases (cytoplasm, present in all M540 bodies) and p53 and DNA (nucleus, present only in a fraction of apoptotic bodies) and by the use of a generic probe for detecting caspases.

PO 052

Reconsidering DNA fragmentation in light of presence of M540 bodies in human semen

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The origin and impact of sperm DNA fragmentation (DF) has been not yet understood. Most studies investigated DF by flow-cytometry, a technique able to measure large number of sperm automatically. The recent identification, within semen, of M540 bodies (round elements virtually lacking of chromatin and occurring at high extent in Oligoasthenoteratozoospermic patients) which are included in the FSC/SSC region characteristic of sperm, raised the question whether they may be confounding in the analysis.

We determined percentages of DF in sub-fertile men by excluding M540 bodies from the analysis by labelling samples with both TUNEL (to detect DF) and propidium iodide (PI, to distinguish sperm from M540 bodies).

The population formed solely by sperm (population A) resulted quite more DNA fragmented than population formed by sperm plus bodies (population B, difference : 8.6 ± 13.9%, mean ± SE; range: from -5.0 to 64.8%). Although testing only 25 subjects, DF in population A correlated with morphology ($r=-0.40$, $p < 0.05$), total motility ($r=-0.36$, $p < 0.05$) and sperm number ($r=-0.37$, $p < 0.05$), at variance with population B (not significant correlations with any parameter). After gating out M540 bodies, we found that PI labelling separated a less fluorescent sperm population (PIdimmer) from a brighter one (PIbrighter) and, mostly important, the latter was entirely DNA fragmented, in all the samples investigated. In addition, the PIdimmer population dramatically correlated with morphology ($r=-0.46$, $p < 0.02$) and total motility ($r=-0.58$, $p < 0.01$) whereas PIbrighter did not.

We conclude that sperm DF can have been underestimated up to now and associated to seminal parameter more closely than suspected. Such association is mainly driven by the incidence of an entirely fragmented subpopulation of sperm. The impact of DF of the other subpopulation has to be entirely investigated. The heterogeneity within DNA fragmented sperm could enlighten the origin of sperm DNA fragmentation.

PO 053

Relationship between DNA fragmentation and ubiquitination in human sperm

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In human sperm, ubiquitination seems to have both positive (for instance in the sperm-oocyte interaction) and negative (marking defective sperm) roles. Indeed, both normal and abnormal sperm result ubiquitinated. Abnormal sperm are often DNA fragmented.

By flow cytometry, we simultaneously detected DNA fragmentation and ubiquitination (the latter by two different antibodies: an IgM against recombinant human and an IgG against rabbit ubiquitin, from here on indicated respectively IgM and IgG). In principle, the technique should distinguish four populations of sperm : A, not ubiquitinated not fragmented; B, not ubiquitinated fragmented ; C, ubiquitinated fragmented and D, ubiquitinated not fragmented.

We found that when the IgM was used, all the fragmented sperm resulted ubiquitinated (i.e population B is lacking) whereas with the IgG no fragmented sperm resulted ubiquitinated (i.e. population C is lacking). This finding suggests that the two antibodies recognise epitopes on different groups of sperm proteins. Such result was confirmed by Western blot analysis: the two antibodies reveal the same protein bands within the range of 7-182 KD, except for some proteins within the MW of 25-49 KD that are not detected by the IgG. Importantly, these data imply that there are ubiquitinated proteins (those recognised by both antibodies) present in sperm only if the cell is not DNA fragmented (population D). In fact, if such proteins were present also in fragmented sperm, they would be recognized by the IgG, but this is not the case.

On the contrary, proteins within 25-49 KD (detected only by IgM) may be present in fragmented (and ubiquitinated according to IgM, population C) and in not fragmented sperm (ubiquitinated according to IgM - population D- but not ubiquitinated according to IgG, population A). Identification of ubiquitinated proteins, to clarify the meaning of DNA fragmentation and the function of bad and good sperm ubiquitination, is underway.

PO 054

Nuclear DNA fragmentation of human spermatozoa

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Objective : The aim of our study was to evaluate the incidence of ejaculated spermatozoa with nuclear DNA strand breaks in patients of Assisted Reproductive Technique Laboratory.

Design : The patients were classified according to WHO standards (1999). Two categories of patients were distinguished: men with normozoospermia (n=26) and men with abnormal sperm parameters (n=68). The latter group included 23 subjects with teratozoospermia, 3 with astenozoospermia, 28 with asthenoteratozoospermia, oligozoospermia, 1 with oligoasthenozoospermia, 5 with oligoterato-zoospermia and 8 cases with oligoasthenoteratozoospermia.

Materials and methods : The sperm DNA strand breaks were identified with the terminal deoxynucleotidyl transferase - mediated dUTP nick end-labeling (TUNEL) assay (APO-BRDU Kit). The TUNEL-positive cells were evaluated in a flow cytometer (FACSCalibur, Becton Dickinson) and in a fluorescence microscope (Axioskop, Carl Zeiss). The semen was examined in JEM-1200 EX (JEOL Ltd) transmission electron microscope.

Results : TUNEL-positive spermatozoa revealed bright green fluorescence in the heads. No significant differences were found in the percentage of sperm with DNA fragmentation between the patients with normozoospermia (9.42±7.68% ; median 6.50%) and with low sperm parameters (14.56±15.51% ; 9.00%).

The proportion of TUNEL-positive sperm was significantly higher in patients with asthenozoospermia (15.10±10.96% ; 12.00% ; 40 out of 94 subjects) and in patients with oligozoospermia (24.93±20.31% ; 20.00% ; 14 out of 94) as compared to normozoospermic men or compared to patients with normal sperm motility (11.69±15.74% ; 6.50% ; 54 out of 94) or to men with normal sperm concentration (11.08±11.50% ; 7.00% ; 80 out of 94) respectively.

A significantly lower percentage of spermatozoa with progressive motility (rapid+slow) was found in patients with >4% TUNEL- positive sperm (71 out of 94) than in patients with ≤4% TUNEL- positive sperm (23 out of 94 men). A significant negative correlation was observed between DNA fragmentation and sperm motility (rs= - 0.38) and between fragmentation and sperm concentration (rs= - 0.33). In electron microscope, a large number of conglomerates containing sperm fragments with cytoplasm and a large number of immature spermatozoa with chromatin and midpiece defects and with excess residual cytoplasm was noted more frequently in subjects with low sperm motility and with >4% TUNEL-positive sperm.

Conclusion : A high proportion of sperm with abnormal genomic integrity may occur in the semen of some men with normal or with low sperm parameters. Our results suggest that sperm DNA fragmentation may be associated with the developmental failure in spermatogenic remodeling process, particularly in patients with low sperm motility. The used test may be considered as an additional assay in evaluation of spermatozoa beside a standard semen analysis and may help to discriminate between fertile and infertile men.

PO 055

Correlation between semen parameters and DNA fragmentation in human spermatozoa

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Objective : The aim of our study was to detect DNA fragmentation in human spermatozoa, in order to investigate a correlation between DNA fragmentation index (DFI) and semen parameters.

Materials and Methods : The patients were divided into two groups according to their semen parameters, 30 patients with asthenozoospermia (group B) and 30 patients with teratozoospermia (group C). The percentage of sperm DNA fragmentation was evaluated by the TUNEL (Terminal deoxynucleotidyl transferase-mediated dUDP nick-end Labelling) assay. Results were compared with those of spermatozoa sampled from 30 healthy men with normal semen profiles (group A).

Results : The difference was not significant between the percentage of DFI in patients with asthenozoospermia and the normozoospermic men (9.46% ± 8.68 and 8.19% ± 6.84, p

not significant). In addition, no significant correlation was found between the motility of spermatozoa and the DFI in the same group.

The patients with teratozoospermia showed a significantly higher percentage of DNA fragmentation than the control group (21.37 ± 17.26% and 8.19% ± 6.84, respectively, P<0.001). There was a positive correlation between abnormal sperm forms and the DFI (r = 0.44, P < 0.01) in the same group (C). The DFI was particularly higher in patients with 91-100% teratozoospermia (group C2) than the other patients with 80-90% teratozoospermia (group C1) (29.55 ± 20.55 versus 14.3 ± 11.01, respectively). The highest percentage of DFI was found among the patients having a high percentage of microcephalic spermatozoa and acrosome anomalies.

Conclusion : Our study noted that impairments of sperm parameters were associated with an increase of DNA fragmentation, this association was strictly related to atypical forms. This finding suggests that teratozoospermia may be the critical sperm parameter associated with hypofertility and when exceeds the proportion of 90 % it would be a prudent sign for analysis of sperm DNA fragmentation.

PO 056

The production of peroxynitrite and proteins nitrotyrosine by human spermatozoa : influence on kinetic sperm features

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Objective : Like all cells living under aerobic conditions, spermatozoa product reactive oxygen species, mosty originating from normal activity. There are many studies to support a role for (superoxide anion) O_2^- and NO^- (nitric oxide) in sperm pathophysiology. Under physiological condition these compounds exist at very low concentration. Peroxynitrite ($ONOO^-$) formed in vivo from O_2^- and NO^- can mediated oxidation, nitration reaction (addition of an NO_2^- group), leading to an impaired function, toxicity and alterations in signalling pathways. In fact $ONOO^-$ reacts rapidly with proteins, lipids and DNA. The nitration reaction gives rise to 3-nitrotyrosine which represents a protein modification specific for $ONOO^-$ formation in vivo : tyrosine nitration is a widely used marker of peroxynitrite. In our study we have determined $ONOO^-$ production in semen and its correlation with kinetic features in spermatozoa, and we set out to determine whether protein tyrosine nitration takes place in the same sample in order to elucidate any pathogenic involvement in sperm cells motility.

Design : Basic study.

Materials and Methods : Semen samples from 29 normal fertile donors (control group) and 69 infertile patients affected by idiopathic asthenozoospermia were analyzed according to WHO 1999 criteria. After liquefaction one aliquot of semen were diluted to 5x106 spermatozoa/mL with Dulbecco's PBS (Phosphate buffer saline) and proxynitrite concentration was measured through the fluorescence of the DCFDA probe. Protein tyrosine nitration was determined with Western ImmunoBlot with an appropriate antibody. Curvilinear velocity (VCL) and straight line velocity (VSL) of sperm cells were determined using a Motion Analysis CASA system.

Results : The control exhibited ONOO- production significantly lower than asthenozoospermic patients (9.11 ± 3.37 vs 27.46 ± 5.77 respectively, p< 0.001) ; furthermore, $ONOO^-$ exhibited a significant inverse correlation with the motility parameters. Moreover, in the western immunoblot there was an increase in the nitration of the tyrosine residues in the asthenozoospermic samples compared to controls.

Conclusions : Althought at level of the whole organism, the reactive chemistry of $ONOO^-$ can be considered beneficial, becaue of its cytotoxicity to bacteria or other invading organism, the formation of protein 3-nitrotyrosine, in vivo, has been shown in a number of inflammatory conditions in human and experimental animals. Our results suggest that higher levels of peroxynitrite are producer in sperm cells of asthenozoospermic infertile when compared to normospermic fertile donors and $ONOO^-$ concentration is inversely related to sperm motility. Tyrosine nitration is enhanced by higher peroxynitrite levels. Thus, a possible pathogenic role in infertile men when asthenozoospermia is the main critical problem may be suggested.

Enzymatic and non-enzymatic measures of oxidative stress in seminal plasma from normozoospermic, asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic males

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Objective : There is growing evidence that damage to spermatozoa by reactive oxygen species (ROS) play a key role in male infertility. The aims of the present study were to assess seminal plasma levels of total antioxidant capacity (TAC) and activities of catalase and superoxide dismutase (SOD) in men with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia compared to normozoospermic males.

Design : A case-control study with simple random sampling was designed. The case group consisted of 46 men with seminal parameters abnormalities that divided into three categories: asthenozoospermic (n=15), asthenoteratozoospermic (n=16) and oligoasthenoteratozoospermic (n=15), according to WHO criteria. The control group consisted of 16 males with normozoospermia.

Materials and Methods : Catalase activity was measured by Aebi spectrophotometeric method. The measurement of SOD activity and TAC was carried out by commercially available colorimetric assays. Differences between groups were assessed using Mann-Whitney U test and Kruskal-Wallis test. Coefficients of correlation were calculated using Spearman's correlation analysis. All hypothesis tests were two-tailed with statistical significance assessed at the p value <0.05 level with 95% confidence intervals

Results : Both catalase activity and TAC levels in all three categories of case group were significantly lower than normozoospermic control males ($p<0.05$). But SOD activity did not show a significant difference between these groups. Both catalase activity and TAC showed a positively significant correlation with sperm motility and normal sperm morphology.

Conclusions : Decreasing seminal plasma antioxidants levels especially catalase activity and TAC could have significant role in etiology of sperm abnormalities.

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Oxidative stress biomarkers levels in seminal plasma from normozoospermic and asthenozoospermic semen samples

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Objective : Poor sperm-forward motility (asthenozoospermia) is considered to contribute to the infertility of a significant number of males, and many cases of decreased sperm motility are not completely understood. One of factors that potentially can cause asthenozoospermia is oxidative stress. The aims of this study were to (i) compare seminal plasma malondialdehyde (MDA), 8-isoprostane, and total homocysteine (tHcy) levels in normozoospermic vs. asthenozoospermic males and to examine their association with sperm motility and also to (ii) investigate the relationship between seminal plasma tHcy levels and lipid peroxidation, as measured by MDA and 8-isoprostane.

Design : We designed a case-control study with a simple random sampling. The case group consisted of 15 asthenozoospermic males. This group was compared with 15 normozoospermic men.

Materials and Methods : Seminal plasma level of 8-isoprostane was measured using enzyme immunoassay (EIA) method. Absorbance was measured at a wavelength of 405 nm using enzyme-linked immunosorbent assay (ELISA) reader and data was presented as pg/ml. The intra-assay coefficient of variation was <10%. The sensitivity and specificity of the 8-isoprostane assay were 5 pg/ml and 100%, respectively. Levels of tHcy were measured using EIA method. Absorbance was measured at a wavelength of 450 nm using ELISA reader. The intra-assay coefficient of variation was <10%. The sensitivity of the tHcy assay was 2.0 µM. MDA levels were determined by the thiobarbituric acid (TBA) assay. The concentration of MDA was expressed as µM. The Mann-Whitney U test was used to compare two groups. Coefficients of correlation were calculated using Spearman's correlation analysis. All hypothesis tests were two-tailed with statistical significance assessed at the p value <0.05 level. The data are expressed as the mean ± SEM.

Results : MDA levels were higher in asthenozoospermic subjects than in control subjects. No differences were seen in 8-isoprostane and tHcy levels in asthenozoospermic subjects and controls. Seminal plasma 8-isoprostane levels showed

an inverse significant correlation with sperm motility and also with normal sperm morphology. Seminal plasma levels of MDA showed an indirect correlation with sperm motility. No relationship was found between MDA and normal sperm morphology. Finally, the correlation between tHys and lipid peroxidation, as measured by MDA and 8-isoprostane, was examined. Seminal plasma levels of tHcy showed no correlation with lipid peroxidation.

Conclusions : Seminal plasma levels of 8-isoprostane and tHcy showed no significant differences between normozoospermic and asthenozoospermic men. Sperm motility correlated inversely with seminal plasma levels of 8-isoprostane and MDA. No relationship was found between tHcy and lipid peroxidation. We also concluded that homocysteine metabolism may not be impaired in asthenozoospermic males. However, a larger sample size is required to confirm these findings.

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PO 059

An investigation of the relationships between sperm morphology parameters, reactive oxygen species production in semen and sperm DNA status

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Objectives : To study the relationship between sperm morphology parameters, the production of reactive oxygen species (ROS) within the spermatozoa and sperm DNA damage.

Design : Prospective analytical study, performed at the Andrology laboratory of the Department of Obstetrics and Gynaecology, University of Jena, Germany.

Materials and methods : Semen samples of 70 men attending the IVF clinic of the Department of Obstetrics and Gynaecology, University of Jena, Germany, were analysed according to the 1999 WHO manual guidelines. In addition, the TUNEL and chromomycin A3 (CMA3) assays were performed and ROS production of spermatozoa was determined by means of the use of dihydroethidine (DHE). Semen smears were stained according to the Papanicolaou method and evaluated according to strict Tygerberg criteria including the acrosome index (AI), detailed classification of specific abnormalities and the calculation of the teratozoospermia index (TZI). The results were analysed with the MedCalc® statistical program for basic descriptive statistics of the resultant semen and functional parameters and rank correlations tests performed.

Results : The mean (±SD) age of the 70 men was 35.1 (±5.8) years. The mean total motility was 57.9% (±19.1) and sperm concentration 51.96 (±51.1) X 106/mL semen. The mean percentage of morphological normal spermatozoa was 3.79% (±2.5), the mean AI was 5.6% (±3.1) and a mean TZI of 1.71 (±0.26) was found. Positive correlations were found between the percentage of TUNEL-negative spermatozoa and the AI ($r = 0.277$; $P = 0.0246$), and the percentage of normal sperm morphology ($r = 0.359$; $P = 0.0036$). Negative correlations were found between the percentage of TUNEL-negative spermatozoa and the percentages of spermatozoa with head defects ($r = -0.349$; $P = 0.0046$), spermatozoa with neck defects ($r = -0.255$; $P = 0.0387$), spermatozoa with small acrosomes ($r = -0.330$; $P = 0.0073$), spermatozoa with tail defects ($r = -0.371$; $P = 0.0026$), the TZI ($r = -0.288$; $P = 0.0194$) and elongated sperm heads ($r = -0.260$; $P = 0.0346$) as well as percentage of live (vital) spermatozoa ($r = 0.6090$; $P < 0.0001$). A negative correlation was also revealed between the percentage of spermatozoa with small acrosomes and sperm viability ($r = -0.2927$; $P = 0.0189$) as well as ROS activity in the ejaculate and sperm viability ($r = -0.3102$; $P = 0.0189$). A strong negative correlation was found between sperm ROS production and CMA3-negative spermatozoa ($r = -0.320$; $P = 0.0083$).

Conclusions : Based on the results obtained in this study it is indicated that normal sperm morphology and normal sperm DNA status (CMA3-negative and TUNEL-negative) are strongly correlated. This supports the idea that problems in the sperm DNA remodelling during chromatin condensation might be one cause of sperm DNA fragmentation. In addition, spermatozoa with small acrosomes seem to be particularly susceptible to ROS activity and therefore present with a low viability, as already indicated in a previous study (Menkveld et al., 2003).

Reference : Menkveld R., El-Garem Y., Schill W-B., Henkel R. : J. Assisted Reprod. Genet.,2003, 20 : 432-438.

Support : Jenapharm, Jena, Germany.

PO 060

The Teratozoospermia Index : A useful sperm morphology parameter for inclusion in the new WHO semen evaluation manual ?

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Objective : The teratozoospermia Index (TZI) was introduced in the 1992 WHO manual as an additional sperm morphology parameter. However, the suggested "normal" reference value of 1.6 was not applicable as this was the value published by Jouannet et al. (1988) for the multiple anomaly index (MAI) based on only three sperm abnormality classes, while the TZI was bases on four sperm abnormality classes. The objective of this study was to perform a literature study investigating the general use of the TZI and find a useable normal reference value based on appropriate data.

Design : A structured literature review study.

Materials and Methods : A PubMed literature search was performed with the word TZI in the title or text as well as available literature for reference to the TZI in their text.

Results : Only three references could be found providing normal cut-off values for the TZI. The first was in the WHO manual for the investigation of the infertile male (Rowe et al., 2000) and the other two by Menkveld et al. (1998, 2001). Rowe et al. (2000) suggested that with a TZI <1.7 successful fertilisation may be expected with IVF and that with a TZI >1.9 ICSI was indicated, but no data or references were given. Menkveld et al. (2001) found a cut-off value of 1.46 when comparing a fertile and subfertile population and a value of 1.64 for in vitro fertilisation based on an oocyte fertilisation rate of >50%. The TZI however, showed the poorest predictive value when compared with normal morphology and the acrosome index.

Conclusions : The TZI as a predictor of male fertility potential is seldom used in the literature and has little predictive value for in vivo or in vitro fertilisation outcome. Therefore, it can be regarded as unnecessary parameter which can be deleted from the new WHO manual.

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PO 061

Sperm morphology assessment: comparison between the manual method (David's criteria) and a computer assisted analysis (Kruger's criteria)

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Objective : Manual morphologic analysis with the David's Classification is used in most French laboratories. Now computer assisted analysis is proposed for objective and reproducible analysis of the spermatozoa morphology. We compared the results of morphological analysis obtained with automatised analysis using Kruger's criteria to those obtained with manual lecture and David's classification.

Materials and Methods : Semen samples were selected from patients referred to our laboratory of reproductive biology for FIV. Analysis was performed both on spermatozoa from ejaculate and on spermatozoa selected (fraction 90%) on Sperm Filter® (cryos, cryo bio system, France).

For the manual analysis, a small drop containing spermatozoa was placed on a slide and a smear was performed. After fixating in a solution of 95% alcohol and 10-15 min air-drying, the slide was stained using the modified Schorr method. The slides were examined under a microscope (400_) and at least 100 spermatozoa were observed.

For the computer assisted analysis, Hamilton Thorne research's system was used. An aliquot of semen was washed and after centrifugation, the resultant pellet, was resuspended to obtain a concentration of 100 10^6/ml. Twenty µl of the sample was spotted on polylysine coated slide and allowed to air-dry at room temperature. The concentration and the droplet size were standardized to produce 10–20 sperm per high-field magnification. Spermatozoa selected on Sperm Filter® gradient were directly spotted on slide.

The air-dried slides were stained with Diff-Quik stain (Merck Diagnostica, Germany). Approximately 100 sperm cells were

evaluated per slide and the percentage of normal sperm morphology, calculated by the computer, was recorded.

Results : To evaluate the reproducibility of the automatised method, coefficients of variation (CV) were determined at the critical steps of the analysis. Measurements were performed - i) after several readings of the same slide, - ii) after readings of several smears of the same sample, -iii) after readings of several preparations of the same semen. No difference in the CV (about 20%, n=4) were observed in these three cases.

The percentage of normal sperm morphology obtained by the two methods were closely correlated R2= 0.51, P<0.01 (n = 33) (Figure 1).

Figure 1: percentage of normal sperm morphology in semen with the manual or automatised methods (n = 33).

A significant correlation was also found in selected spermatozoa R2= 0.54, P<0.01 (n = 10).

Conclusion : The main factor of variability for this analysis is linked to the slides reading. The application of computer-assisted technology for the evaluation of sperm morphology may have reached a level of accuracy and precision that makes it acceptable for routine application and it is less time consuming. These results indicate that Hamilton Thorne research's system might be used as an initial screening test for the evaluation of sperm morphology in order to make decisions in planning strategies for the treatment of infertile couples.

PO 062

Teratozoospermia : clinico- laboratorial analysis

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One of the most mass manifestations of disturbance of spermatogenesis is a teratozoospermia (TZ). Its reasons however are unknown. The purpose of this study became revealing possible factors leading development TZ. For this purpose 572 men (mean age 32,3±7,1 years) with TZ and 120 men (mean age 32,4±7,6 years) with normozoospermia (NZ) as the control are surveyed.

Average height, weight, BMI also as well as average total testicular volume (26,0±4,3 vs 26,4±4,3) in both groups had shown significantly no differences. There was no significant difference among volume (3,7±1,7 vs 3,5±1,4 ml) and pH (7,7±0,4 vs 7,6±0,9) of the ejaculates. Average of sperm concentration in men with TZ (78,7±76,2x10^6/ml) has appeared considerably smaller than in men with NZ (121,8±81,7x10^6/ml, p=0,000). At absence of distinctions in parameters of sperm motility (a) 25,8±10,5% vs 27,2±10,3%, b) 39,5±9,4% vs 41,0±11,1%, c) 11,0±5.5% vs 10,3±5,7%, d) 24,0±10,8% vs 21,9±9,0%), and their viability (72,7±11,1% vs 75,8±7,0%).

The percentage of spermatozoa with normal morphology was 13,5±8,2% in TZ group and 37,2±8,1% (p=0,000) in NZ group. There are significant differences in morphologically modified spermatozoa with a small (7,7±8,6% vs 4,6±4,5%, p=0,000), cigar-shaped (17,1±12,5% vs 5,2±3,8%, p=0,000), pear-shaped (11,5±9,2% vs 4,5±3,9%, p=0,000), double head (1,0±1,7% vs 0,5±1,0%, p=0,001), defective neck (11,6±6,6% vs 6,9±3,8%, p=0,000) and tail defects (8,5±5,9% vs 5,3±3,2%, p=0,000). The average maintenance of leukocytes in the ejaculate had no significantly differences between the men with TZ and NZ (0,5±3,0 vs 0,3±1,3x106/ml).

Frequency of occurrence left-side (18,4% vs 14,2%) and bilateral (16,1% vs 17,5%) varicocele in men with TZ and NZ has appeared approximately identical, as well as frequency of occurrence bilateral (47,2% vs 42,0%) and unilateral left-side (13,3% vs 11,7%) testicle hypotrophy.

There also was no significant difference in chronic prostatitis (22,9% vs 21,0%) while total frequency of detectability sexually transmitted infections were significantly 1.4 high in comparison with TZ group.

At the same time TZ group has significantly high frequency of inflammatory parotitis (20,5% vs 1,7%, p=0,000), infectious hepatitis A (9,6% vs 0,0%, p=0,001), acute orchitis (3,3% vs 0,8%), surgical operation on account of left-side varicocele (8,2% vs 0,0%, p=0,002), chronic tonsillitis (3,7% vs 0,8%) and acute appendicitis (17,8% vs 10,1%, p=0,000. in the anamnesis.

While in a NZ group were more often observed unilateral epididymo-orchitis(12,6% vs 3,9%, p=0,000), trauma of scrotum (16,0% vs 4,6%, p=0,000), infectious hepatitis B(12,6% vs 3,0%, p=0,000), surgical operation on account of hemorrhoids (17,7% vs 0,5%, p=0,000), adenoids (10,1% vs 2,5%, p=0,000) and inguinal hernias (12,6% vs 8,6%). Besides, correlation dependence is found out between sperm concentration and percentage of spermatozoa with a normal structure (r=0,29, p=0,000) in men with TZ.

Thus, higher maintenance of spermatozoa with a small, cigar-shaped, pear-shaped, double head, defective neck and tail defects comes to light in men with TZ. Secondly, it is possible to carry a number of the transmitted infection-inflammatory diseases bacterio-viral etiology as rule extragenital localizations to the factors potentially leading to TZ. Thirdly, value of prostate inflammatory diseases, chronic sexually transmitted infections and varicocele in genesis of TZ is minimal.

Relationship between human sperm morphology and acrosomal function

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Objective : In this study, we investigated the relationship between functionality of the acrosome and sperm morphology.

Design : Acrosome reaction (AR) was separately determined in live and dead sperm and in those with normal, small, and large sized acrosomes by means of the triple stain.

Material and Methods : In 50 patients, triple stain was performed before and after induction of acrosome reaction. Sperm samples were washed with human tubal fluid medium containing 1% serum albumin and split into 2 aliquots for test and control. IN live and dead spermatozoa the percentage of acrosome-reacted cells was separately determined in those with normal sized, small and large acrosomes. Morphology was analyzed according to strict criteria after Papanicolaou stain.

Results : AR and morphology correlated regarding detection of large and small sized acrosomes, but not for normal sized acrosomes. Spontaneous AR was significantly influenced by acrosomal size. Sperm with large (11.4%) and normal (9.2%) acrosomes exhibited a significantly higher percentage of life spontaneously acrosome-reacted sperm than those with small acrosomes (4.5%). Sperm with small acrosomes were associated with a higher percentage of cell death.

Conclusion : The results indicate that sperm with small acrosomes are more susceptible to cell death and non-physiological acrosomal loss. Acrosome size reflects the physiological capability of sperm function and therefore male fertility potential.

In vitro beneficial effect of date seed oil extract supplementation on human spermatozoa acrosome reaction

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Objective : Date seed oil (DSO) is a natural mixture of antioxidants containing polyphenols and α tocopherols. The aim of this study was to test the antioxidants effect of DSO on human spermatozoa acrosome reaction assessed in vitro, before and after oxidative stress induced by hydrogen peroxide (H_2O_2).

Design : Prospective study.

Setting : Laboratory of histology, Faculty of medicine in Sfax, Tunisia.

Materials and Methods : The study was carried out on motile spermatozoa (spz) selected from infertile sperm samples (n=16) by a discontinuous gradient method, using sill select (selection medium) and ferticult (culture medium). DSO was obtained by lipid extraction protocol according to Besbes and al [1] and dissolved in no toxic Gum Arabic. After selection, motile spz were incubated in CO2 (5%) at 37°C for 3 hours, necessary time for spontaneous acrosome reaction; then, four experimental situations were tested: E1 (motile spz in ferticult added with 0.01mM H2O2 alone); E2 (spz in ferticult added with 0.01% DSO alone), E3 (spz in ferticult added initially with 0.01 % DSO then with 0.01mM H_2O_2, 30 minutes later) and us control situation: E4 (spz in ferticult alone). After that, all the preparations were allowed in CO_2 (5%) in 37°C for 30 min, then used for the determination of reacted acrosome (RA) spz percentages by an immunofluorescence method, using flurescein isothiocynate FITC-PSA according to Cross and al [2].

Results: We found that the percentage of RA spz in the presence of DSO was higher than in the situation without DSO (control) and that it was reduced in the presence of H_2O_2, but without reaching signification (figure1). However, we noted that in E3 experimental situation (DSO and H_2O_2), the percentage of the RA spz was significantly higher than in E1 situation (H_2O_2 alone).

Conclusion : Our results led to suggest that DSO has beneficial effects on spermatozoa functions, and enhances their fertilization capacity. So we can propose the use of the

purified DSO "in vivo" or "in vitro" treatment essays in male infertility.

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PO 065

Tyrosine phosphorylation patterns in human sperm incubated in HTF media or bound to solid state hyalutronic acid

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Introduction : During spermiogenesis there is a sperm plasma membrane remodeling that facilitates the formation of zona pellucida and hyaluronic acid (HA) binding sites. Upon binding to zona pellucida, human sperm undergo capacitation-related changes. Sperm with arrested maturation can fail to bind to the zona pellucida and HA. Also, the sperm selection attributes of HA and zona pellucida is similar: HA-bound sperm show no cytoplasmic retention, persistent histones or DNA fragmentation. Previous studies have shown that specific protein tyrosine phosphorylation (TP) changes in spermatozoa are related to events associated with capacitation and zona-binding. In the present study, we have studied TP patterns in spermatozoa isolated from media or bound to HA.

Materials and Methods : Immunofluorescence was performed with antisera for TP. We prepared sperm smears, HA- and zona-bound sperm from each semen sample suspended in HTF medium (Irvine Scientific) and using HA-coated slides (MidAtlantic Diagnostics). The sperm were fixed with 3.7% formaldehyde after 0 time and 4hr incubations. The slides were treated with a monoclonal anti-phosphotyrosine antibody (Sigma). These steps were followed with FITC labeling for sperm assessment with fluorescence microscopy. Data analyses were carried out with SigmaStat (Jandel, CA). All values are mean±SEM.

Results : We determined the TP pattern in different regions of sperm. TP occurred in the equatorial segment, acrosomal area, neck, and principal piece of sperm. The extent of TP significantly increased at 0 and 4 hours. We observed major TP changes, related to elapsed time and HA-binding, only in the neck region and principal piece of sperm. The % sperm with neck and principal piece phosphorylation in HTF at 0 time and 4 hours were: 14.4±5.3 and 41.5±8.8 (p=0.02, N=3777 sperm) compared to 21.9%±4.9% and 51.5±5.5% (p=0.01, N=3555 sperm) in the HA-bound sperm fraction, respectively.

Conclusions : The capacitation-related pattern of TP in different regions of the sperm increased in a time-related fashion. The extent of TP in the sperm neck and principal piece, a pattern that is characteristic for marker of sperm activation, increases with time and contacts with HA. Furthermore, it is of interest that the sperm TP pattern changes are similar in sperm that are bound to HA or to zona pellucida of oocytes.

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PO 066

Toluidine Blue image analysis cytometry for the prognosis of male fertility potential

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Objective : It is well known that the conventional semen analysis can be used only for the approximate prediction of the male fertility potential. Numerous studies have sought a key factor(s) that would be highly predictive, including also sperm acrosome status, cell membrane integrity, sperm DNA integrity, etc. However, to date, no single laboratory test can precisely assess a man's fertility potential. Hereby we introduce the new approach to use the Toluidine Blue (TB) test for sperm chromatin conformation assessment as a highly predictive test for the evaluation of the male fertility.

TB test is able to distinguish (a) sperm nuclei with normal chromatin conformation and normal DNA integrity (light blue

cells), (b) sperms with DNA double-strand breaks and loosely packaged chromatin (dark purple cells), (c) two "intermediate" conditions - light purple and intensive blue sperm cell nuclei. Results of the TB test (proportion of the dark purple cells) correlates with those from SCSA and TUNEL assays.

Materials and Methods : Sperm samples from 21 sperm bank donors and 42 infertile men from barren couples with excluded female factor were subjected to the conventional semen analysis and TB image analysis cytometry test as described earlier. ROC curve analysis was used to set the threshold between fertile and infertile men. Three different data sets were exploited in this analysis: (a) concentration of TB-light blue cells/mL ejaculate (assuming both TB-test results and sperm concentration) ; (b) proportion of TB-light blue cells (sperm concentration not assumed); (c) sperm concentration only (thresholds of 5, 20 and 40 $x10^6$/mL were tested).

Results : When threshold between fertile and infertile men was set at 28 millions TB-light blue cells/mL ejaculate, it possessed high sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). These values were considerably lower when only the TB-data or sperm concentration were considered alone (Table).

	TB-light blue cells x 10^6/mL	TB-light blue cells %	Sperm conc. x 10^6/mL		
Threshold	28	35	5	20	40
Sensitivity%	93	77	21	54	72
Specificity%	86	86	100	100	90
PPV%	93	92	100	100	94
NPV%	86	64	27	51	61

Conclusions : Hereby we suggest simple and easy accessible test with high precision for male fertility potential assessment. TB test is cheap and easy to perform, and highly correlative with alternative tests like SCSA and TUNEL, requiring more expensive equipment and reagents.

Evaluation of innovative assays in routine semen analysis

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Objective : Standard semen analysis does not provide information about subcellular processes in human sperm. For a better understanding of spermatozoal defects not monitored by routine semen analyses new fluorescence based assays were introduced. It was our aim to determine the stability of the fluorescence signals of these recently developed tests in the course of time for estimation of practicability.

Design : Prospective study.

Materials and Methods : Semen samples from healthy donors (n=87) were separated in 4 aliquots to perform the following assays : 1. detection of active Caspase-3 (FLICA™, Immunochemistry Technologies), 2. analysis of integrity of the mitochondrial membrane potential, MMP (Mitosensor™, Clontech), 3. detection of externalization of phosphatidylserine, EPS (FITC-labeled, monoclonal mouse anti-human phosphatidylserine antibody, clone 1H6, Upstate Cell Signaling Solutions) and 4. detection of CD46 (monoclonal, FITC-labeled mouse anti-human CD46, IgG2a, Biomeda). Afterwards, paraformaldehyd 4% was added to all aliquots. The fluorescence of each sample was evaluated by flow cytometry (FACS Calibur, Beckton Dickinson) at day 0, day 3, day 7, day 10 and day 14.

Results : Variances up to ± 5% positive spermatozoa from the value measured at day 0 were estimated as acceptable deviation. The Caspase-3 FLICA™ showed mean variances < 5% at day 3, 7 and 10. At day 14 the mean difference was 7.6%. In contrast, the Mitosensor™ and the EPS detection showed variances > 5% already at day 3. The CD46-FITC labeling displayed absolute variances < 5% CD46 positive spermatozoa at day 3, 7, 10 and 14.

Conclusions : Although immediate analysis of the fluorescence signals is recommended, it is possible to evaluate activation of Caspase-3 up to 10 days after the staining in human spermatozoa. Labeling with CD46-FITC on the spermatozoal surface gives very stable fluorescence signals and flow cytometry analysis can be performed at least within the following 2 weeks. The FACS evaluation of MMP integrity and EPS detection should be conducted at the same day.

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SCSA parameters and sperm preparation for IVF

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Objective : The routine examination of semen does not identify defects in sperm chromatin structure. Evaluation of raw sperm DNA damage appears to be a useful tool for assessing male fertility potential both in vivo and in vitro. Human semen is heterogeneous in quality within a single ejaculate. Thus in IVF, it is important to use a sperm processing technique that enables the recovery of a concentrated and highly functional sperm population. A study was performed to examine the effect of semen processing by density gradient on human sperm DNA integrity.

Design : Descriptive and correlational clinical study.

Setting : This study was performed in the University Hospital of Tours, France.

Materials and Methods : Semen samples were collected from 236 patients undergoing IVF in our centre and processed by density gradient. For each sample (before and after processing), DNA fragmentation (DFI) was recorded with the use of Sperm Chromatin Structure Assay (SCSA). Controls were used to determine intra (n = 20) and inter (n = 63) variability (both of 5%) of the SCSA in our hands. The difference between DFI before and after density gradient allowed us to divide patients in three groups: Group 1 without difference (n = 37), Group 2 with decreased DFI (n = 181) and Group 3 with increased DFI (n = 18).

Results : The clinical pregnancy rate was lower (11.1%) in the Group 3 with an increased DFI after sperm processing by density gradient compared to Group 1 and 2 (24.3% and 24.9%, respectively). Moreover Group 3 was also associated with the lowest sperm parameters (numeration and motility) both before and after density gradient.

Conclusion : Most studies investigating SCSA parameters used neat semen with conflicting results. Our data show DFI alteration during sperm processing among IVF patients and suggest that combining DFI assessment before and after density gradient might be more accurate. Such results support the necessity to revalidate SCSA in sperm processed for use in IVF.

Sperm Chromatin Structure Assay (SCSA) predicts the outcome of ART

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Objectives : Since the introduction of assisted reproduction techniques (ART) there has been a continuous search for markers with the potential to predict a couple's chance of obtaining a pregnancy and to be used for improving the relatively low baby-take-home rates, which have been held stable (20-30%) during the last two decades. During recent years there has been an increased focus on the role of sperm chromatin integrity in relation to fertility and several sperm DNA integrity testing methods are now available. One of these tests, the Sperm Chromatin Structure Assay (SCSA) has been suggested to be a powerful predictor of fertility in vivo as well as in vitro. The available data, however, have been based on limited numbers of treatment cycles. The aim of this study was to assess the clinical role of SCSA, in IVF, ICSI and IUI, based on a large study material.

Design : Prospective study

Materials and Methods : 998 consecutive couples undergoing (ART) were included. Intrauterine insemination with partner's sperms (IUI) was performed in 387, IVF in 388 and ICSI in 223 cases. SCSA results were expressed as DNA Fractionation Index (DFI) and High DNA Stainable cell fractions (HDS). Reproductive outcome parameters were clinical pregnancy (CP) and delivery (D).

Results : For IUI, the odds ratios (ORs) for CP and D were significantly lower in the group with DFI > 30% as compared to those with DFI> 30% (95% confidence intervals (CI): 0.10 (0.02-0.42) and 0.07 (0.01-0.48), for CP and D respectively) (Fig 1). No statistical difference between the outcomes of IVF vs. ICSI was observed in the group with DFI £ 30%. In the DFI > 30% group, however, the results of ICSI were significantly better than those of IVF. Comparing ICSI to IVF, the ORs (95% CI) for CP and D was 3.0 (1.4-6.2) and 2.0 (1.0-4.5), respectively. Neither sperm concentration nor motility could add to the prediction of the treatment outcome.

Conclusions : Our study shows that DFI can be used as an independent predictor of pregnancy and birth in couples

undergoing IUI. A high DFI does not exclude successful treatment, but the OR of pregnancy was 3 times higher by ICSI than by IVF when the DFI exceeded the level of 30%. When DFI exceeds 30%, ICSI should be the method of choice, also in cases where traditional sperm parameters are normal.

Figure1 : Odds ratios (OR) for Biochemical pregnancy (BP) of intrauterine insemination (IUI) treatment in relation to cut off level for the DNA Fragmentation Index (DFI).

PO 070

Sperm annexin V binding, mitochondrial membrane potential and DNA fragmentation changes in predicting IVF and ICSI outcome

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Background : We determined the value of sperm membrane phospholipid asymmetry, mitochondrial membrane potential (MMP) and DNA fragmentation changes in predicting the outcome of classical IVF and ICSI.

Methods : Prospectively we evaluated sperm of men of infertile couples (n=248) on the basis of spermiogram. After sperm preparation using gradient centrifugation, apoptosis markers were assessed by flow cytometric analysis: apoptotic and necrotic cells by use of PI and annexin V, MMP by means of DiOC6(3) staining and DNA fragmentation after addition of acridine orange. One to 6 months later, 45 and 51 couples were involved in 50 IVF and 64 ICSI cycles, respectively. The percentage of necrotic cells, the DNA fragmentation index and the percentage of spermatozoa with high MMP were correlated with fertilization rate (less or more than 25% of fertilized oocytes), presence of at least one blastocyst, all embryos arrested, and occurrence of a clinical pregnancy. Data were analyzed by logistic regression and analysis of variance.

Results : In IVF, sperm concentration and necrotic cells were significantly related to fertilization rate (40.53 ± 6.57 vs 74.48 ± 7.97x106 spermatozoa per mL, P=0.038 and 30.07 ± 18.77 ± 2.39%, P=0.040, respectively). More necrotic cells were found in couples not obtaining at least one blastocyst or developing only arrested embryos (27.15 ± 3.73 vs 20.18 ± 2.78%, P=0.03 and 34.83 ± 3.79 vs 18.70 ± 2.40%, P=0.026). Increased sperm concentration was predictive of the occurrence of a clinical pregnancy (95.60 ± 17.98 vs 54.47 ± 5.48x106 spermatozoa per mL, P=0.04).

In ICSI, a higher MMP predicted a higher fertilization rate (53.10 ± 3.38 vs 40.50 ± 9.74%, P=0.016). Fewer necrotic cells, higher MMP and younger males predicted the development of at least one blastocyst (33.00 ± 3.24 vs 43.50 ± 5.86%, P=0.039, 57.51 ± 3.46 vs 39.21 ± 5.92%, P=0.039 and 34.1 ± 0.7 vs 37.3 ± 1.2 years, P=0.036). Older male age predicted the arrest of all embryos (40.1 ± 2.1 vs 34.7 ± 0.7 years, P=0.036). Neither spermiogram nor apoptosis markers could predict a pregnancy.

Conclusion : Beside male age and sperm concentration, annexin V and MMP changes, which are early indicators of apoptosis, may predict the outcome of classical IVF and ICSI.

PO 071

Apoptosis signaling in spermatozoa after standard semen preparation in an IVF clinic

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Objective : The inclusion of apoptotic sperm during assisted reproductive techniques (ART) may be one of the reasons for suboptimal success rates. The aim of our study was to evaluate the potential of routine standard semen preparation in the IVF laboratory to eliminate spermatozoa with activated apoptosis signaling from patients semen samples prior to ART.

Design : Prospective study.

Material and Methods : Semen samples from 20 infertility patients scheduled for ART (IVF and ICSI procedures) were investigated after informed consent. Following density gradient centrifugation (DGC) and swim up small aliquots were taken from each sample to analyze the levels of Caspase-3 activation (CP3) using carboxyfluorescein derivatives (FLICA™) and the integrity of the mitochondrial membrane potential (MMP) using a lipophilic cationic dye (MitoSensor™) by flow cytometry. In addition progressive motility was observed according WHO. Aliquots from the neat semen served as controls.

Results : Standard semen preparation by DGC and swim up resulted in improvement of motility (WHO a+b, Mean±SD : from 29.5±10.4 to 73.0±8.8%, p<0.01). Semen samples of patients

contained 46.2±17.7% spermatozoa with intact MMP and 51.8±14.9% spermatozoa with active CP3. After DGC + swim up the amount of MMP intact sperm was increased to 78.5±11.8% ($p<0.01$), while the percentage of spermatozoa containing active CP3 was reduced to 26.1±15.0% ($p<0.01$). However, there were inter-individual differences in the separation effect: Minimal reduction of sperm with disrupted MMP and active CP3 was 6.0% and 0.7% respectively, maximum reduction was observed as 65.5% (disrupted MMP) and 49.3% (CP3).

Conclusion : Semen samples of subfertile patients contain significantly higher levels of spermatozoa with disrupted MMP and activated CP3 when compared to results of previous studies on fertile donors. Although there was a reduction in the majority of the samples, further reduction might be achieved by recently developed molecular separation methods combining the standard semen preparation and Annexin-V-based elimination of apoptotic spermatozoa.

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PO 072

Comparative study of two semen processing techniques in idiopathic male infertility

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Objective : To compare two commonly used techniques of semen processing: swim-up (SU) (Using Ham's F10, Biochrom A.G, Berlin, Germany) and density gradient centrifugation (DGC) (Using Sil-select Plus, Ferti-Pro N.V., Beernem-Belgium).

Design : Prospective experimental study.

Settings : The Department of Dermatology and Andrology, Assiut University Hospital.

Patients : A group of men diagnosed to have idiopathic male infertility.

Methods : The study included 44 semen-processing trials for separate semen samples from men with idiopathic male infertility using SU in 23 samples, and DGC in the other 21 samples.

Main outcome measure : Comparison between SU and DGC in the recovery of higher numbers and percentages of motile morphologically normal sperm in debris free media.

Results : Total sperm recovery percentage was 33.88±11.37 with DGC and 13.22 ± 4.02 with SU ($P < 0.001$). Percentage of increase in progressive motility % was 46.79 ± 24.98 with DGC and 147.41 ± 58.94 with SU ($P < 0.001$). Percentage of increase in total motility % was 36.57 ± 30.88 with DGC and 84.10 ± 58 with SU ($P < 0.001$). Total motile sperm (TMSC) recovery % was 44.92 ± 12.44 with DGC and 25.28 ± 5.81 with SU ($P < 0.001$). Percentage of reduction in round cell number (RCs)/HPF was 64.51 ± 18.83 with DGC and 90.22 ± 14.87 with SU ($P < 0.001$). Percentage of increase in normal morphology % was 27.87 ± 11.30 with DGC and 26.93 ± 11.49 with SU ($P > 0.05$).

Conclusions : DGC is superior to swim-up in the recovery of higher number of motile sperm. It can be used in dealing with low quality semen samples especially in the presence of oligozoospermia or low native total motile sperm count. SU is superior to DGC in improving the percentage of motile sperm. So, It can be used in processing semen samples with poor motility percentages and relatively good sperm count.

PO 073

Improvement of sperm characteristics after sperm preparation by buoyant density gradient

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Objective : of our study is to estimate the interests of sperm preparation technique by swim up on pressure gradient of pure sperm on sperm characteristics at patients with morphologically abnormal semen samples within the framework of coverage of male or mixed infertility.

Design and place : Service of Cytogenetic and Biology of Reproduction, University Hospital F Hached Sousse, Tunisia.

Methods : This study included 53 infertile patients presenting at least to the spermogram a hight percentage of abnormal forms ($\geq$ 70%) according to the WHO criteria. The sperm of the patients was obtained by masturbation after 3 days of sexual abstinence. The initial examination of the sperm contains an evaluation of the following parameters: volume, initial motility, percentage of abnormal forms. Then the sperm benefits from a centrifugation on 3 layer pure sperm gradient (90%, 70%, 45%) followed by a wash of the 90% layer in another environment of culture.

Results : The morphological analysis of spermatozoa after centrifugation showed a significant increase of the average percentage of the normal forms which doubles and pass from 18.4% to 36.13%. The indication of multiple abnormalities (IAM) is improved in a significant way ($p < 0,000$) passing from 1.6 to 1.38. Also the percentage of sperm cells has microcephalic, disentangled heads; the abnormalities of the acrosome double tails, anguled and short spermatozoa are significantly lowered.

Conclusion : The sperm preparation by centrifugation on pressure gradient of pure sperm allows a quantitative and qualitative selection of spermatozoa. This technique allows us of to improve spermatozoa power fertilizing and obtaining embryos to be able to evolutionary mattering in case of appeal to reproduction medical care.

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PO 074

Prospective cross-over trial comparing the results of intrauterine insemination with and without mild ovarian stimulation and timed natural intercourse in male and unexplained subfertility

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Objective : Although the effectiveness of intrauterine insemination (IUI) plus controlled ovarian hyperstimulation (COH) with gonadotrophins is well established in couples with unexplained infertility, it carries the risk of ovarian hyperstimulation and multiple pregnancy. Limited evidence exists on the effectiveness of IUI with a milder stimulation with clomiphene citrate (CC). Furthermore, whether the IUI with COH is more effective than IUI without COH in the male subfertility is controversial. The aim of this study was to compare the effectiveness of IUI with and without mild ovarian stimulation in male and unexplained subfertility.

Design : A prospective cross over trial.

Materials and Methods : Seventy-eight couples (duration of infertility:median=3.9 years; female age: median=35.5 years) with unexplained or male factor subfertility without ovulatory dysfunction and other causes of subfertility in the female were recluted. They were randomly assigned to sequential timed natural intercourse (NI), IUI and IUI + sequential CC and human menopausal gonadotrophin (HMG) repeated 3 times, unless pregnancy occurred.

Results : In this preliminary analysis the results of 326 observed cycles were evaluated. The clinical pregnancy rate (PR)/cycle was 1.6% in NI, 6.5% in IUI, 8% in IUI+COH (risk ratio=5; 95% CI: 1.3-18.9 vs NI). A trend toward higher PR was observed with IUI+COH than in IUI (11.4% vs 6.7%) in cycles with normal semen, but not in cycles with abnormal semen (motile sperm count $<10x10^6$/mL and/or normal morphology <15%, and/or MAR test >50%), where PR was 8% in IUI+COH and 7.1% in IUI, excluding cycles where recovered motile spermatozoa were $< 1x10^6$. Teratozoospermia strongly affected the IUI outcome: PR=2/84 cycles (2.4%) vs 13/101 cycles (12.9%; risk ratio=5.4; 95% CI: 1.5-19.3). In oligo/asthenozoospermia without teratozoospermia, the PR/cycle was 6/43 (14%).The highest PR was achieved in the male immunological infertility (4/26 cycles; 15.4%).

Conclusions : This preliminary analysis indicates the effectiveness of IUI with a mild stimulation with sequential CC and HMG in infertile couples with unexplained infertility, while IUI without COH appears to be equally effective in the male subfertility, thereby representing a proper first-choice therapy, when $>1x10^6$ motile sperm are recovered and teratozoospermia is excluded.

PO 075

Percutaneous epididymal and testicular sperm obtaining procedures for intracytoplasmic sperm injection - 10 years experiences in Fertility Clinic "Novum" (1996-2005)

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Objectives : About three years after Palermo's report [„Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte", The Lancet 1992;340;17] the

first ICSI (Intracytoplasmic Sperm Injection) procedure in Poland was done in January 1995, in Fertility Clinic "Novum", Warsaw. Next, in the same center, the first ICSI with percutaneously sperm obtained from epididymis (ICSI-PESA, Percutaneous Sperm Aspiration) was performed in April 1996 and also the first procedure with testicular sperm (ICSI-TESA, Testicular Sperm Aspiration) was done in September 1996.

Design : This report presents 10-years experience in percutaneous sperm obtaining for ICSI-PESA/TESA procedures in "Novum".

Materials and Methods : Azoospermic men (mean age 32 y.o.) from infertile couples were qualified for sperm retrieval procedure as follows: with obstructive azoospermia from epididymis (PESA) and with non-obstructive azoospermia from testis (TESA). All procedures were done ambulatory, under general anesthesia (short IV : Propofol and Fentanyl). In PESA, both epididymides were punctured percutaneously using syringe 2 mL with fine needle No 5, then aspirated liquid was examined under light microscope for sperm presence for ICSI-PESA. In TESA, testicular tissue from both testes was obtained using 1.6-mm needle (Hepafix® set, B.Braun, Melsungen, Germany) and technique tissue preparations reported by Schoysman ["Modern sperm retrieval techniques and their usefulness in oocyte fertilization " BJU Int. 2001.88.141] were performed for ICSI-TESA. Since 1999, during regular diagnostic testicular biopsy in each patient, scheduled cryopreservation and storage of epididymal liquid and part of testicular tissue were done (the rest of specimen's part was fixed in Bouin's solution for routine histological evaluation). This procedure made possible to avoid next testicular biopsies during future ICSI. Mean time of one procedure was 15-20 minutes. After 2 hours of observation each patient was verified by anesthesiologist and urologist and left center. During 2-3 days after procedure commons analgesic drugs (e.g. Paracetamol) and scrotal ice compresses were applied; no antibiotics were used as a rule.

Results : These final results were stated by parents' declarations up to the end 2005 (incomplete unfortunately, because all childbirths were outside of "Novum").

1996 – 2005 (Novum, since 1995 No in all : ICSI-5635, pregnancies-2007)		**ICSI-PESA**	**ICSI-TESA**
Pregnancy		74	57
Children (including twins)		48 (10)	35 (12)
Delivery :	cesarean section	26	16
	spontaneous	12	19
Birth weight (g) :	single	2070-4230	2250-4130
	twins	1720-2860	1810-2580
Spontaneous abortions or pregnancy terminations (No women)		9	7

In men from both groups no important complications after sperm obtaining and no prolonged breaks in life activity (work, study and sex) were reported.

Conclusions : Application sperm obtaining from epididymides and testes in micromanipulation procedures IVF/ICSI-PESA, TESA makes real possibility to be fathered for men with obstructive and non-obstructive azoospermia. Percutaneous sperm obtaining is non-complicated, safe, relatively cheap minimal invasive procedure.

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PO 076

Evaluation of Motile Sperm Organelle Morphology Examination in unselected In Vitro Fertilization with Intra-Cytoplasmic Sperm Injection

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As many teams, we were interested by the results of the method of high-magnification sperm observation and selection developed by Bartoov [1] and proposed for ICSI failure specific indication. We wished to evaluate the technique in unselected ICSI.

Objective : The aim of the study was to evaluate the relevance of Motile Sperm Organelle Morphology Examination (MSOME) compared with usual selection made in ICSI. We determined, among conventionally selected sperm for ICSI, how many appeared abnormal when observed with high magnification and assessed the predictive value of this parameter on unselected ICSI outcome.

Materials and Methods : The study included 25 successive unselected ICSI attempts in the IVF Laboratory of the Hospital of Poissy (France) and 5 controls. ICSI were performed according to usual protocols used in the laboratory. Twenty five motile spermatozoa of the migrated fraction, still available after ICSI, and "injectable" according to conventional morphology assessment in ICSI ("normal" or "as normal as possible" with magnification of x200-400) were assessed by MSOME (superior to x4500) and classified according to criteria adapted from Bartoov's work [2] and taking into account David's sperm morphology classification [3]. We compared the results of MSOME, the results of conventional sperm morphology analysis and ICSI results.

Results : In this small series of ICSI with diverse indications, we found very high frequencies of abnormalities (over 70 % vs 46.4% in controls, $p<0.0001$), particularly nuclear vacuoles.

The rates of anomalies detected with MSOME and conventional morphology assessment were correlated, excepted for some of them i.e. nuclear vacuoles. No predictive value of the morphology of sperm assessed with high magnification (including vacuoles) was found for fertilization rate, embryo quality and ICSI outcome. At the opposite of previous reports, pregnancies were obtained with very abnormal sperms. In this series of unselected ICSI, nuclear vacuoles do not seem to have pejorative meaning for pregnancy outcome.

Conclusions : Our work leads to several perspectives. It would be interesting to understand the "anatomical" support of vacuoles observed with MSOME and their meaning. The question of the phenotype-genotype relation, that is to say the possible correlation between sperm morphology and genetic content, could be investigated, for example by studying FISH and MSOME, Tunel and MSOME. Finally, a prospective analysis should be performed in clearly defined indications to validate the potential applications of the method for high-magnification sperm observation and selection.

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PO 077

The fate of paternal mitochondria in non-human primate embryos

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Objective : Sperm-derived mitochondria are integrated into the oocyte at fertilization but seem to disappear during the early cleavage phases of mammalian embryos. The developmental potential of embryos seems to be closely related to their ability to destroy these mitochondria, but the mechanisms underlying their loss of function are not yet understood. By staining the mitochondria prior to fertilization the fate of the paternal mitochondria can be followed in the embryo and distinguished from the much bigger maternal mitochondrial pool.

Design : Prospective animal in vitro study.

Materials and Methods : To study the fate of the paternal mitochondria we chose a non-human primate model, Callithrix jacchus. We implemented a hyperstimulation protocol to increase the number of oocytes collected. Penile stimulation of the male marmosets was utilized to obtain motile sperm. The mitochondria of the sperm were stained with different vital dyes which are integrated into the mitochondrial membrane. After in vitro fertilization the developing embryos were cultured and fixed at different cleavage points to track the sperm-derived mitochondria. Embryos were stained for markers involved in apoptotic or mitochondrial degeneration such as cytochrome c, APAF-1; Bcl-2 or ubiquitin. By utilizing immunofluorescence or laser-scanning microscopy the paternal mitochondria were located in the blastomeres of the developing marmoset embryos. Live imaging of the fluorescence indicator was also used.

Results : We hyperstimulated 39 female marmosets in 81 cycles and obtained 580 oocytes. Paternal mitochondria could be found in all fertilized embryos up to the 8-cell-stage shortly before the transition into the morula stage. Although the cytochrome c remained in the sperm-derived mitochondria up to this stage, the function of the paternal mitochondria appeared to be lost. The JC-1 stain indicated that these were not functional sperm-derived mitochondria. Marmoset sperm were clearly labeled for ubiquitin, a destruction pathway marker. However after fertilization ubiquitin did not colocalize with the sperm-derived mitochondria and had disappeared.

Conclusions : The paternal mitochondria remained functional up to the 8-cell-stage and there was no indication that the early embryo has a specific mechanism for dismantling them. Mitochondrial destruction does not appear to be initiated by known mechanisms such as the apoptotic or the ubiquitin pathway. If the developing primate embryo destroys these mitochondria it must occur after the 8-cell-stage. Paternal mitochondria are distributed into all blastomeres of the very early embryo, which may lead to their segregation into trophoblast cells which do not contribute to the embryo. This would explain why no paternal mitochondrial DNA is found in any species analyzed.

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Achievement of twin pregnancy with spermatozoa from a globozoospermic man after ICSI treatement

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Globozoospermia is an uncommon severe form of teratozoospermia characterized by round-headed sperm with an absence of acrosomes. Family cases of globozoopermia suggest that this pathology has genetic origins, but the mode of inheritance remains unknown. So far, no responsible genes have been identified. Pregnancies and live birth are rarely reported even with ICSI treatment.

Objective : We report a successful achievement of a twin pregnancy in a Tunisian couple in which the male partner had globozoospermia and referred to us for cytogenetic evaluation.

Methods and Results : A 35 year old man and his 24 year old partner, a consanguineous couple sought assisted conception after 2 years of primary infertility. Semen sample revealed the following characteristics : volume 4 ml ; sperm concentration 121.2x106/ml ; 25 percent progressive motility at 30 min and 65 percent vitality. All the spermatozoa were round-headed. Hormonal profile was within normal range. The karyotypes of the two partners were normal. The couple proceeded, after a first unsuccessful cycle of ICSI, to a second cycle of ICSI in a Belgium centre. Of 12 oocytes retrieved, 11 were micro-injected and 6 fertilized normally. Four compacting embryos with eight or more cells were obtained. Two embryos were transferred 72 h after micro-injection and two others were cryopreserved for future use. A twin clinical pregnancy results and culminates in a spontaneous vaginal delivery of live twin, a female and a male. The infant's birth weight was respectively 2.3 and 2 kg. The twin show normal development after 6 months.

Conclusion : Genetic counselling was very hard in this situation. In fact, if some genetic causes of male infertility have been identified and are clearly explained to patients, many others genetic factors of infertility remain largely unexplored.

Non-steroidal anti-inflammatory drugs and plasma steroids in male athletes

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Objective : Arachidonic acid (AA) metabolites modulate the activity of the hypothalamus-pituitary-gonadal (HPG) and adrenal (HPA) axis. The aim of the present preliminary investigation was to evaluate if a short-term treatment with acetylsalicylic acid (ASA), an inhibitor of prostaglandin (PG) synthesis, in usual clinical conditions might influence male athletes' resting plasma steroid hormones concentration.

Design : We evaluated the effects of a ten-day treatment with ASA on morning plasma free-testosterone, total testosterone (T), dehydroepiandrosterone sulphate (DHEAS) and cortisol (F) concentrations in moderately trained males caucasian athletes affected by minor exercise-related muscle-skeletal traumas.

Materials and Methods : Morning plasma free-T, T, F, DHEAS and their ratios were evaluated immediately before and after the administration of ASA 800 mg two times a day for ten days, in twelve subjects with the same baseline characteristics : chronological age of 19-21 years, height 169.5-193.5 cm, weight 62.2-85.1 kg, VO2max 51.2±7.9 $ml^{-1}min^{-1}$ kg^{-1}. As a control, we evaluated the same volunteers after five/six weeks of wash-out from ASA administration. For the experimental control evaluations, the volunteers took placebo twice a day for ten days and suspended physical exercise for the same period of time all the volunteers suspended their physical training during both ASA or placebo treatment.

For therapeutic purposes, the athletes were treated with an anti-inflammatory therapy and with a ten day break from physical exercise.

Results : Treatment and time conditions showed a significant interaction effect on F values, and the comparison between treatments revealed that morning mean plasma F concentration was significantly lower after ASA treatment than after placebo treatment (577.2±98.1 $mmol.L^{-1}$ vs 588.2±102.6 $mmol.L^{-1}$, $p = 0.023$). We showed significant differences between ASA and placebo treatments, both for ΔF (-42.5±171.3 and -17.4±156.8, respectively, $p=0.045$), and for $\Delta F\%$ (-0.08±26.5% and 2.83±25.5%, respectively, $p=0.04$).

It is of clinical interest the marginal interaction effect observed for T concentrations after ASA treatment, close to statistical significance (from 24.1±3.9 nmol.L^{-1} to 28.1±8.2 nmol.L^{-1}, p = 0.052). The statistical evaluation of T variations suggested an effect of ASA treatment in increasing plasma T concentrations. In fact, we showed significant differences between ASA and placebo treatments, both for ΔT (4.1±5.1 and 0.7±1.2, respectively, p=0.047) and for ΔT% (15.0±20.2% and 2±5.3%, respectively, p=0.049). It is of great interest that whereas after placebo plasma T variations ranged from -4.8% to 8.5%, with respect to pre-treatment values, after ASA treatment this range varied from -7.7% to 44.2%.

Conclusions : In the present study we observed that, in comparison with placebo, a short-term course of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in moderately trained athletes is able to influence the resting plasma steroid milieu. Whereas our previous data suggested the involvement of PGs in the neuro-endocrine responses to physical stress, the hypothesis that NSAIDs treatment may act on hormone pathways secretory activity and/or metabolism also in resting conditions is quite intriguing, despite the high variability of the evaluated parameters and the number of volunteers.

PO 080

In men with coronary disease both estradiol and free testosterone may predispose toward atherogenic lipid profile but higher blood level of total testosterone is associated with a fewer critical coronary stenoses

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Objectives : Estrogen receptors are present in the cardiovascular system and in men with inherited inactivating mutations of genes encoding estrogen receptors the occurrence of premature coronary artery disease (CAD) was documented, indicating preventive role of estrogen for CAD in men. However, elevated plasma estrogens have been found in men surviving myocardial infarction, suggesting that hyperestrogenemia may be a coronary risk factor. Here we aimed to assess the correlations between blood levels of sex steroid hormones or lipid profile and degree of coronary arteries stenoses in men with CAD.

Methods : 111 men with stable CAD, aged 36-73 yrs, underwent elective coronary angiography. Dissemination and degree of coronary stenosis were assessed using 3 angiographic indices. Total cholesterol (T-Ch), high density lipoproteins cholesterol (HDL-Ch), low density lipoproteins cholesterol (LDL-Ch) and triglicerydes (TG) were estimated in a single blood sample. To avoid influence of short-term fluctuations of the hormones, testosterone, estradiol, dehydroepiandrosterone sulfate and sex hormone binding globulin (SHBG) were measured on the basis of two subsequent blood samples taken with 30-min. interval. These two samples were mixed together for a single determinations of each hormone. A free testosterone index (FTI) was calculated as a quotient of total testosterone (nmol/l) and SHBG blood levels (nmol/l) multiplied by 100.

Results :

I) Positive, significant correlations were found between blood level of estradiol and T-Ch (r=0.29, p<0.01), between estradiol and LDL-Ch (r=0.34, p<0.005) as well as between FTI and LDL-Ch (r=0.23, p<0.05). Blood level of estradiol negatively correlated with HDL-Ch/T-Ch ratio (r= -0.21, p<0.05).

II) Blood level of T-Ch positively correlated with an index of the dissemination of coronary artery stenoses, represented by sum of all narrowings in 15 coronary artery segments (r=0.26, p<0.05). In turn, blood level of total testosterone negatively correlated with an index that represented a number of critical stenoses (over 50% of artery lumen occlusion) (r= -0.26, p<0.05).

Conclusions :

1) In men with CAD, blood plasma estradiol level is predictive for T-Ch and LDL-Ch concentrations and for HDL-Ch/TCh ratio, while FTI for LDL-Ch, indicating that estradiol and bioavailable testosterone may predispose toward atherogenic lipid profile.

2) Higher blood level of total testosterone is predictive for fewer number of the critcal coronary artery stenoses, indicating that in men testosterone may have a beneficial effect on the coronary arteries, irrespectively to the relation between FTI and LDL-Ch.

3) Higher blood level of T-Ch is predictive for higher total number of disseminated coronary artery stenoses supporting the concept of an involvement of T-Ch in the pathogenesis of CAD.

PO 081

Testicular dystenesis syndrome and estrogen receptor alpha polymorphisms : association with sperm production and cryptorchidism

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Objective : Although the importance of estrogens in male reproduction is indisputable, little attention has been paid to the role of Estrogen Receptors (ERs) gene mutations in male infertility. The aim of the present study was to get insights into the role of ER alpha in spermatogenesis and cryptorchidism through the analysis of polymorphic sites in the ER alpha gene : i) (TA)n repeat allelic variant in the promoter region; ii) five SNPs in distal introns. A previous study on the Italian population found a significant correlation between the (TA)n repeat variant and lumbar bone mineral density indicating that allelic combinations with higher number of (TA)n repeats are functionally more active. As far as the five distal SNPs are concerned, a specific haplotype "AGATA" was recently described as a risk factor for cryptorchidism in Japanese men. In this ethnic group SNP12 was the tag SNP for the AGATA haplotype.

Materials and Methods : (TA)n repeats : a large group of infertile and normospermic men (n=347) was studied by using an automated sequencer by GeneScan software. Each size of the PCR products was subjected to direct sequencing on the autosequencer for the definition of the correct TA repeats length.

AGATA haplotype and SNP12 : a large group of patients (n=335) and controls (n=567) of two Caucasian populations (Italian and Spanish) was analyzed. The genotyping was performed by direct bidirectional sequencing using an automated sequencer.

Results : (TA)n repeats : Although the (TA)n polymorphism failed to show a significant association with male infertility, we found a significant effect of this polymorphism on sperm count. In the group of infertile men the mean TA repeats number and sperm concentration and total sperm number were inversely correlated, showing an association between higher TA repeat number (genotype A) and lower sperm production. In line with this observation, normospermic subjects with genotype A had a significantly lower mean sperm concentration ($p<0.05$) and a lower total sperm count ($p<0.01$) with respect to men bearing genotypes with shorter TA alleles.

AGATA haplotype and SNP12 : We confirmed that SNP12 is the tag SNP for the AGATA haplotype also in Caucasians. No association between SNP12 and spermatogenic disturbances was found in the Italian and Spanish populations. However, in contrast with the Japanese population, we found a significant protective effect (OR=0.5) for ESR1 SNP12 on cryptorchidism.

Conclusions : Our data indicate that specific allelic combinations of the ER α which confer a stronger estrogen effect, may influence negatively human spermatogenesis. A plausible explanation would be that not only deficit of estrogens but also an exaggerated estrogen action related to this genetic variant (eventually combined with environmental factors), can be deleterious. Whether the observed negative effect is the expression of a disturbance in the early testis development or in the adult testis remain to be established.

With regard to SNP12 polymorphism, the discrepancy between our and the Japanese study may be related to genuine ethnic differences and/or different environmental conditions. Therefore, the observed associations (although with opposite effect) with criptorchidism encourage future studies on independent cases and controls from different ethnic and geographic origin.

PO 082

Environmental risk factors for testicular cancer

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Objective : Numerous research studies have examined the question of an increased incidence of testicular cancer over the last 50 years, although its causes are as yet little known. The environment is a major axis of the search for risk factors of testicular cancer. Some specific occupational exposures

have been suggested, but their attributable risk is too low to account for all the cases observed. According to the present environmental hypothesis, two exposure windows may intervene in the genesis of testicular cancer : in utero during the development of male gonads, and later during adult life. In order to target more closely the risk factors of testicular cancer, an etiological case-control study was carried out on these two exposure windows during male life.

Design – Materials and Methods : The data analysed were taken from the Reprhom study, which involved 229 patients with testicular cancer and 800 partners of pregnant women.

Results : Concerning male exposure in utero, the fact that the mother had had a garden (possible exposure to pesticides), had lived near a factory (possible exposure to pollutants) or taken treatment during pregnancy (possible exposure to exogenous hormones), increased the risk of development of testicular cancer in her son. Concerning exposure during adult life, certain specific occupational exposures (use of glue, metallurgy) could be considered as risk factors, but the latter findings should be interpreted with caution as exposure was recorded only for the previous three months.

Conclusion : Several previously unknown risk factors for testicular cancer have emerged, notably intra-uterine factors, contributing towards the postulate that endocrine disturbances may partly explain the recent marked increase in testicular cancer.

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PO 083

Impact on survival of diagnostic delay in testis cancer

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Introduction : Testis cancer (TC) has become a common cancer, whose incidence is rising especially in young men. Although the cure rate is 95%, a group with bad prognosis continues to be resistant to treatment. Diagnostic delay is defined as the time elapsing from the onset of tumour symptoms to the day of diagnosis. We aimed to assess the relationship between diagnostic delay, disease stage and survival rate, and to examine trends in diagnostic delay over the last 20 years.

Design : Regional population-based study

Materials and Methods : Diagnostic delay was investigated by mailed questionnaire in 542 patients diagnosed with TC between 1983 and 2002 in the Midi-Pyrenees region, France. Information regarding disease, treatments and follow-up was obtained through their medical records. We analysed diagnostic delay according to histological type, stage and survival, and studied trends in diagnostic delay.

Results : Mean diagnostic delay was 3.7 +/- 5.1 months and was longer in seminoma (4.9 +/- 6.1 months) than in non-seminomatous germ-cell tumour (NSGCT) (2.8 +/- 4.0 months). Diagnostic delay was significantly linked with disease stage and the 5-year survival rate in the whole population and in NSGCT. Duration of diagnostic delay did not change significantly over the study period.

Conclusion : Diagnostic delay remains highly correlated with stage and survival, in particular for NSGCT. Programmes to enhance awareness and knowledge of TC are recommended in order to diminish the diagnostic delay. Measures that may be taken to reduce diagnostic delay are notably campaigns for TC awareness and testicular self-examination.

PO 084

Epidemiology, prognostic and management of pure teratoma of the testis

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Objectives : To study prognostic and management of pure teratoma of the testis.

Design : Multicenter and regional population-based study.

Materials and Methods : A retrospective multicenter study was performed using (1) the data base of the Midi-Pyrenees

region and (2) of the Military Hospital of Val de Grâce. Among more than one thousand patients diagnosed between 1987 and 2003, 20 had a pure teratoma of the testis. All orchiectomy specimens were examined by 2 uro-pathologists. All patients were contacted to perform physical examination and CT scan.

Results : Diagnosis was confirmed in 17 patients, non teratomatous components were identified in the remaining 3 patients.

Among the 8 patients with stage I pure teratoma, 4 received adjuvant chemotherapy and 4 were entered in a surveillance program. With a mean follow up of 105 months, Teratoma Growing Syndrom occurred in 1 patient and controlateral seminoma in one patient. In 2005, all the patients were disease free.

The remaining 9 patients with metastatic teratomas were managed with chemotherapy followed by resection of residual tumor masses. With a mean follow-up of 149 months, all the patients were disease free.

Conclusion : Pure teratoma of the testis are rare tumors (4% of all non seminomatous germ cell tumors). Their histological diagnosis is difficult and needs careful examination. Their prognosis is excellent. Surveillance after orchiectomy should be proposed in the absence of vascular emboli. Complete surgical resection of retroperitoneal tumor masses is mandatory in metastatic forms.

PO 085

Sperm DNA integrity in men treated for childhood cancer

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Objective : The treatment of childhood cancer has greatly improved, and today approximately 80% of the patients are cured. The clinical challenge of today is therefore not only to cure, but also to cure at minimal cost. Oncological treatment of a growing child is associated with several long-term complications, of which persistent damage to the reproductive function is one. Men treated for childhood cancer are expected to suffer from decreased fertility. Furthermore, cancer treatment implies a potential risk of sperm DNA damage although data on sperm DNA integrity in childhood cancer survivors are sparse. The aim of this study was to assess DNA Fragmentation Index (DFI) as an indicator of sperm DNA integrity in survivors of childhood cancer. Sperm DNA integrity is of clinical importance both in terms of fertility and the potential genetic risk for the offspring of the father treated for cancer.

Design : The study was based on a patient cohort of 97 male childhood cancer survivors. They had all received treatment for a malignant disease in childhood or adolescence during the period 1970-1989. Patients were asked to deliver a semen sample, and sperm DNA integrity was assessed with two methods, Sperm Chromatin Structure Assay (SCSA) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).

Materials and Methods : In the cohort of 97 men 32 chose to participate. Due to azoospermia (n=7) or inability to deliver a sample, semen from 22 men were eligible for DNA assessment .All samples were analysed with SCSA and 19 were analysed with TUNEL. Mean age at diagnosis of the 22 men was 9 years, and at the time of semen delivery 36 years. Of the 22 patients 19 were treated with either radiotherapy or the combination of both radio-and chemotherapy. As controls served 278 military conscripts.

Sperm DNA integrity was assessed by SCSA, a method based on the phenomenon that chromatin with abundant DNA double strand breaks has a tendency to denaturate when exposed to acid-detergent, whereas normal chromatin remains stable. The extent of DNA denaturability is expressed as the DNA Fragmentation Index (SCSADFI). DFI expresses the proportion of cells containing denaturated DNA. Using flow cytometry a total of 5000 cells were analysed. The TUNEL assay quantifies the incorporation of fluorescently labeled dUTP at breaks of double-stranded DNA. Using flow cytometry a total of 10000 cells were analysed.

Results : In men treated for childhood cancer, TUNELDFI was significantly higher than for controls (means: 17.7% vs 12.7%; p=0.045). SCSADFI was also higher in the cancer group, but without reaching statistical significance (means: 17.3% vs 13.6%; p=0.072).

Conclusions : We have demonstrated that childhood cancer treatment induces permanent sperm DNA damage. However, many questions remain. To what extent is the fertility potential affected in male survivors of childhood cancer with preserved spermatogenesis ? Are there any genetic risks for the offspring of these men, especially considering that these men are probably prone to an increased need of assisted reproduction ? Finally, in order to evaluate the impact of different treatment modalities, i.e. radiotherapy and different chemotherapeutic drugs, a larger material is required.

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PO 086

Semen cryopreservation and testicular cancer : analysis of 1158 patients in the French Federation of CECOS

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Objective and Design : Testicular cancer is one of the most common pathologies observed in young males aged between 20 and 35 years (12% of cancers). Sperm quality at the time of the diagnosis is a major parameter able to modify spermatozoa resistance to the process of freezing and thawing. Various studies have reported a decline in semen quality before therapy in testis cancer depending on the histological feature of tumour, but not confirmed in all published data. The objective of our study was to analyse retrospectively, within 11 CECOS (Centre d'Etude et de Conservation des Œufs et du Sperme Humain), a population of men who presented a testis cancer and who consulted for sperm cryopreservation between January 1, 1999 and December 31, 2003. and to examine (i) their medical history (ii) their fertility prior to cancer diagnosis (iii) the pre-freeze and post-thaw semen quality before and after orchidectomy as well as (iv) the effect of histological features and cancer stage on semen parameters.

Patients and Methods : A population of 1158 patients was recruited, before or after orchidectomy but before initiating a treatment by chemo- and/or radiotherapy. Age, urological history and fertility at the time of the diagnosis were retained for each patient. Semen samples were evaluated after liquefaction according to the World Health Organization. Sperm parameters taken into consideration were : volume (mL), sperm concentration (10^6/mL), total sperm count (10^6), motility (a+b : %), morphology (% of typical forms), number of straws per sample, post-thaw motility (a+b : %) and total number of forward motile spermatozoa per straw (10^6). The histological diagnosis as well as the evolutionary stage of the disease were required for each patient : The statistical analysis was carried out by Gecem society (Service of Biometrics, Montrouge, France) with a financial support of FARO 2005 (Fond d'Aide à la Recherche Organon).

Results : A total of 1158 patients aged between 14 and 56 years (30 years ± 7), presenting a pure seminoma (S: 48%), a mixed tumour (MT : 33%) or a non seminoma tumour (NST°: 19%) including 13.6% embryonal carcinoma (EC) and 3.8% teratoma (TE). The majority of the tumours were at stage I (71%), 19% were at stage II and 10% at stage III of Boden. A quarter of the patients (26%) presented urogenital history, cryptorchidism being the most frequent (14%). Patients with seminoma were oldest ($p<0.001$) but also most fertile. Patients with urogenital history (including cryptorchism) were a little less fertile, moreover, their initial sperm parameters were more altered. The semen samples were carried out before (28%), after (58%), before and after (13%) orchidectomy. A decrease of total sperm count was observed in 43% of the patients as well a reduced motility in 81% of them. Sperm concentration and total sperm count were significantly lower before orchidectomy for S and MT compared to EC ($p<0.001$), decreased after orchidetomy for NST (for EC, $17x10^6$ before vs $87x10^6$ afterwards) and did not vary for S. These sperm parameters are more altered in the tumours of stage III ($p=0.0008$). Initial and post-thaw motility significantly decreased in patients with cryptorchidism ($p<0.001$) or non proven fertility ($p=0.007$).

Conclusion : This series of testis cancers is the most important one reported in the literature and calls in question the data previously published. Indeed, seminoma may alter spermatogenesis more significantly than the NST (including EC). Seminoma seems to completely inhibit spermatogenesis in the testis carrying the tumour.

PO 087

Testicular sperm cryopreservation at the time of removal of testes and subsequent intracytoplasmic sperm injection in patients with testicular cancer

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Objective : Testicular cancer is a disease increasing in incidence in developed countries. It attacks many young men who do not have a child yet or would like to have more children after efficient cancer treatment.

Design : The aim of this study was to evaluate, whether the program of testicular sperm cryopreservation could be set up at the time of removal of one or both testes in patients who did not cryopreserve sperm (ejaculate) before starting cancer treatment and expressed their wish to do so.

Material and Methods : When removal of one or both testes (orchiectomy) in 9 patients was performed by urologists, a part of healthy tissue from the removed testis was preserved and transported in a pre-warmed, sterile Flushing Medium (MediCult, Denmark) to the IVF laboratory. Approximately 1 hour later it was checked for the presence of spermatozoa under inverted microscope, frozen in a 20% cryoprotectant glycerol solution, using a controlled-rate cooling machine, and stored in liquid nitrogen at -1960C. At the time of use it was thawed at room temperature and intracytoplasmic sperm injection (ICSI) was performed using testicular spermatozoa.

Results : The mean age of 9 patients at the time of biopsy was 30 years. It ranged from 26 to 54 years. Eight patients were younger than 30 years. All patients had seminoma tumors in one or in both testes. In 8 patients one testis was removed because of the tumor; another testis has already been removed in the past or was abnormal. In 1 patient both testes were removed. In 8 patients motile spermatozoa were found in testicular tissue. In one patient the testicular tissue was thawed 9 years after cryopreservation and 15 oocytes of 32-years-old female partner were microinjected with testicular spermatozoa using ICSI. Seven embryos developed and two, both at the blastocyst stage after extended culture, were transferred into the uterus. Female partner conceived and gave birth to a healthy child. In 8 patients testicular tissue is still stored after 1 to 10 years after cryopreservation.

Conclusions : In a big proportion of patients with seminoma testicular cancer motile testicular sperms can be found at the time of the testes removal which may be cryopreserved for later use. This offers the testicular cancer patients good chances to father a child in the future, using ICSI, and means an enormous psychological support them.

Support : We would like to thank to all urologists, Department of Urology, to the whole team of gynaecologists, andrologists, and embryologists of our Department, and to Ms. Mojca Pirc, B.Sc.

Cryopreservation of testicular tissue from prepubertal mice

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Introduction : The increased number of childhood cancer survivors has focused attention on sequels of treatment, more specifically on gonad damage and dysfunction. Cryopreservation of ejaculated spermatozoa should be proposed for sexually mature boys. However, when boys failed to collect ejaculated semen samples, or for prepubertal boys, testicular biopsy and cryopreservation of (i) isolated testicular spermatozoa when possible or (ii) testicular tissue should be proposed. However, it is absolutely necessary to establish an optimal procedure for freezing and thawing immature testicular tissue before application in humans. In the present study, we evaluated viability, induction of apoptosis and morphology of immature testicular tissue after different cryopreservation protocols in a prepubertal mouse model.

Materials and Methods : Immature testis obtained from six-day-old CD1 mice (Charles River, France) were cryopreserved using several protocols with either 1,2-propanediol (PrOH) or dimethyl-sulphoxide (DMSO) as cryoprotectants with a final concentration of 1,5 M combined with different cooling rate curves [(i) controlled slow protocol with and (ii) without seeding, (iii) controlled quick protocol and (iv) uncontrolled protocol]. In the controlled protocol, the cooling rate was regulated by a Minicool 40 PC (Air Liquide Santé, France). In the uncontrolled protocol, the samples were placed 24 or 48 hours at -80°C in a freezer before being plunged into liquid nitrogen. Eight testis were used for each condition and a minimum of three independent series of experiments was performed for each condition. Cell viability was determined by a trypan blue and propidium iodide staining. Apoptosis markers [Annexin V, terminal deoxynucleotidyl transferase assay (Tunel)] were evaluated immediately after thawing and isolation of germ cells using an enzymatic treatment as well as 24 hours after culture of germ cell suspension at 33°C. We determined the recovering of testicular cells after thawing (RTC : 10^6 per 100mg of testicular tissue) and the rate of

alive and non apoptotic germ cells (RAA : %) in the different conditions. Morphological evaluation of seminiferous tubules was also performed, establishing a scoring (ST) of alterations affecting tubule architecture, cytoplasm and nuclei of germ cells and somatic cells (Sertoli and Leydig cells). ST varied from 0 (normal morphology) to 10 (major morphological alterations).

Results : For DMSO and PrOH, a controlled and slow cooling rate curve offered the best condition of cryopreservation. However, RGC (58 millions per 100mg for DMSO vs 42 millions for PrOH) was the most satisfying with DMSO in a controlled and slow cooling rate curve without seeding, the same result was observed for RAA (89% for DMSO vs 62% for PrOH). Furthermore, this protocol preserved the architecture of seminiferous tubules (ST = 0.7). Therefore, severe alterations of seminiferous tubules were observed in protocol using PrOH with controlled and quick cooling rate curve (ST = 8.2). DMSO gave the best results in the different cooling rate curve tested compared to PrOH.

Conclusion : In the different cooling rate curves, DMSO proved to maintain, after cryopreservation, not solely immature testicular tissue architecture but also viability of germ cells and somatic cells, better than PrOH. Substantial modifications in the cryopreservation procedure should be performed for PrOH.

PO 089

Subcellular localization and regulation of type 5 phosphodiesterase in coropra cavernosa cells

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Objective : In adult animals, smooth muscle cells (SMC) rest in a quiescent phenotype and different stimuli trigger the shift from non-proliferating to proliferative phenotype. Proliferation and contraction are both regulated by the intracellular second messenger cAMP and cGMP, which are degraded by phosphodiesterases (PDEs). The most abundant PDE present in corpora cavernosa is the erectolytic cGMP specific type 5 PDE, the molecular target of sildenafil. We have previously demonstrate that PDE5 is expressed prevalently in the cytoplasm and in particular in spots corresponding to centrosomes using human myometrial cells (Dolci, S. et al. Biochem Biophys Res Commun 341:837, 2006). The centrosomal localization suggested us to investigate the possibility that PDE5 levels might be related to cell cycle activity of SMC.

Materials and Methods : In this study we used cultures of rat corpora cavernosa (CC) cells obtained modifying the methods of Krall et al. (Krall, J.F. et al. Biol Reprod 39:913, 1988). RNA and protein extracted from CC cells were utilized for RT-PCR analysis and for western blot using a specific PDE5 antibody. Immunohistochemistry were performed to study the localization of PDE5 in adult CC cells.

Results : In rat CC cells we see the same localization of PDE5 observed in miometrial cells. Thus we studied the modulation of PDE5 mRNA and protein in conditions in which the proliferation was inhibited. Indeed, we observed, after 72 hours of serum starvation, a significant increase of PDE5 mRNA levels in adult rat CC (0,027±0,008 v.s. 0,050±0,010; $p>0,05$). This result was confirmed by western blot where, in the same condition, an increase of about 50% of PDE5 proteins levels was found. A similar result was observed when the proliferation rate of cells was blocked with the MEK inhibitor U0126 that has been demonstrated to block the proliferation of SMC. By using an RT-PCR semi-quantitative assay we observed an increase of PDE5 mRNA levels from 0,034±0,018 within basal to 0,074±0,026 within U0126 treated cells ($p>0,05$). We also show that this 100% increase of PDE5 expression can be reversed by the addiction of serum in 24 hours. In fact, a dramatic down regulation of PDE5 mRNA from 0,050±0,010 to 0,0154±0,0037 ($p>0,02$) has been found when serum was added to cells previously starved for 72 hours. Platelet-derived growth factor (PDGF) was demonstrated to promote proliferation of SMC. We observed a significant down regulation of PDE5 RNA expression in CC60 cells after 24 hours treatment with PDGF ($1,40x10^{-1}$±0,018 v.s. $3,98x10^{-2}$±0,015 ; $p>0,02$) and this effect was completely reversed by U0126 treatment.

Conclusion : These results suggest that expression of PDE5 is correlated with the progression of cell cycle and demonstrated that PDE5 is not only the main regulator of penile erection but can be also considered a marker of contractile, non-proliferating phenotype of corpora cavernosa SMC.

Effect of sildenafil administration on penile hypoxia induced by cavernous neurotomy in the rat

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Objectives : Radical prostatectomy is an effective therapy for men with clinically localized prostate cancer. A significant number of men develop erectile dysfunction after radical prostatectomy (PPED) due to intraoperatory cavernous nerve injury causing hypoxia and fibrosis of corpus cavernosus. We established an experimental model of bilateral cavernous neurotomy (BCN) in the rat in order to investigate whether sildenafil treatment in PPED patients could prevent penile tissue damage.

Design and Methods : One, 5 and 10 days after neurotomy, animals were treated or not with a single dose of sildenafil (25mg/kg orally) one hour before sacrifice. To analyze penile oxygenation, rats of each experimental group received (one hour before sacrifice) an intraperitoneal injection of the bio-reductive drug pimonidazole hydrochloride (hypoxyprobeTM-1, 60 mg/Kg), which has been recognized as a standard marker for in vivo imaging and quantification of hypoxia.

Results : With immunohistochemistry for hypoxiprobeTM, we found that BCN induced massive hypoxia at all times investigated in corpora cavernosa sections from the experimental rats, as revealed by computer-assisted quantitative image analysis. This tissue hypo-oxygenation was significantly reduced in sections from sildenafil treated rats at 1 and 5 days after neurotomy, while at 10 days this reduction was less evident and not significant. In addition, functional studies indicated that hypoxic corpora cavernosa tissues were hypersensitive to the relaxant effect of the endothelin receptor type B (ETB) agonist IRL-1620, due to the previously described hypoxia-induced overexpression of ETB receptors. Accordingly, ETB mRNA expression (real time RT-PCR) was significantly increased in corpora cavernosa from BCN rats, and was restored to control levels by sildenafil administration at all times investigated.

Conclusions : our results indicate that sildenafil treatment can positively influence penile tissue oxygenation after cavernous nerve injury, with its effect being more evident the earlier it is administered.

Differential effects of two-week treatment with atorvastatin of elocalcitol, two RHOA/ROK signalling modulators, on erectile function and sildenafil responsiveness in spontaneously hypertensive rats

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Objective : Increased RhoA/Rho-kinase (ROK) signalling is known to impair erectile function. Since it has been recently described that spontaneously hypertensive rats (SHR) over-express penile RhoA and are unresponsive to phosphodiesterase 5 (PDE5) inhibition (Wilkes N et al. Int J Impot Res, 2004; 16:187), we tested treatments known to inhibit RhoA activation, on erectile function and sildenafil responsiveness in SHR.

Design and Methods : SHR (10-weeks old) have been treated for two weeks with atorvastatin (5 and 30 mg/Kg/day), or with elocalcitol (30 mg/Kg/day), a non-hypercalcemic vitamin D receptor (VDR) agonist. The normotensive Wistar Kyoto (WKY) rats have been used as controls. Erectile function was evaluated as frequency-dependent intracavernous pressure/mean arterial pressure (ICP/MAP) ratio after electrical stimulation (ES) of the cavernous nerve. Gene expression analysis was performed by real-time RT-PCR.

Results : at the selected concentrations, neither atorvastatin affected cholesterol, nor elocalcitol affected calcaemia in both SHR and WKY rats. In WKY, sildenafil (25 mg/Kg by oral gavage) greatly increased ICP/MAP ratio after ES of the cavernous nerve, while in SHR, both basal and sildenafil-stimulated ICP/MAP ratio were depressed. Atorvastatin did not affect basal ICP/MAP at any concentration tested. However, it dose-dependently increased sildenafil effect on ES-induced penile erection, which was significantly potentiated by 30 mg/Kg dosing. Morever, at this dose, atorvastatin normalized the over-expression of RhoA mRNA observed in SHR, without affecting the expression of other genes such as ROK1, ROK2, PDE5, nNOS, and eNOS. Conversely, elocalcitol, at a dose known to ameliorate bladder overactivity by inhibiting RhoA activation (Morelli et al., Prostate, 2006 in press), failed to restore depressed ICP/MAP ratio, sildenafil responsiveness and RhoA overexpression in SHR. Gene expression analysis

showed that SHR rats expressed elevated levels of VDR mRNA in the bladder (almost 5-fold increase over WKY), but not in the penis.

Conclusions : our data confirm that erectile function and sildenafil responsiveness are impaired in SHR because of an increased RhoA signalling. Atorvastatin, at a dose unable to affect lipids, ameliorates sildenafil effectiveness and down-regulates RhoA expression. Conversely, elocalcitol was ineffective in restoring erectile function in SHR, either alone or in combination with sildenafil. The differential quantitative expression of VDR in bladder and corpora cavernosa suggests a plausible mechanism for the tissue-specific effect of elocalcitol on RhoA/ROK contractile pathway.

PO 092

Erectile function after brachytherapy for localised prostate cancer : long term results

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Objective : To evaluate erectile function before and after (125)I brachytherapy for localised prostate cancer using a validated patient-administered questionnaire (IIEF-5).

Design : Prospective non-randomised study with self-administered questionnaire.

Materials and Methods : A total of 316 patients with preimplant erectile function (EF) assessed using the International Index of Erectile Function (IIEF-5) questionnaire underwent permanent implantation of (125)I seeds between July 2000 and May 2006 for localised prostate cancer. No patient received supplemental external beam radiation therapy ; 29% received adjuvant antiandrogen therapy for up to 4 months. Of the 316 patients, 210 (67%) completed and returned the questionnaire. Fourteen patients who were not sexually active were excluded. Mean age was 66 years (43-80). Mean follow-up was 4 years (2-6). The questionnaire focused on EF without PDE5 inhibitors. EF was compared before and after brachytherapy and potential confounding factors (age at diagnosis, age of partner, diabetes, hormonal treatment, initial erectile status, dosimetry parameters) were analysed by univariate and multivariate analysis.

Results : Before treatment, 81 (41%) patients had no erectile dysfunction (ED) (score 22-25); 53 (27%) had mild (score 17-21), 38 (19%) mild to moderate (score 12-16), 11 (6%) moderate (score 8-11) and 13 (7%) had severe ED (score 1-7). Overall, 172 (87%) had a score above 12.

After treatment, 21 (13%) patients had no ED ; 26 (16%) had mild, 46 (27%) mild to moderate, 24 (14%) moderate and 51 (30%) had severe ED. Overall, 93 (55%) had a score above 12.

Among the 172 patients who had a preimplant score above 12, after treatment 19 men (11%) had ceased to be sexually active, 34 (20%) remained in the same category as before, 43 (25%) deteriorated by one category, 72 (42%) by two or more, and 4 (2%) patients improved. The median time to the onset of ED was 8 months. In multivariate analysis, preimplant erectile function was the best predictor of brachytherapy-related EF ($p<0.0005$).

Conclusions : Most of the population treated by brachytherapy was sexually active before treatment and had no or mild preimplant ED. The proportion of patients with no or mild ED decreased from 87% before to 55% after treatment. The quality of preimplant erectile function is the best predictor of post-treatment erectile function.

Support : None.

PO 093

Erectile function after short antiandrogen therapy for localised prostate cancer in sexually active men

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Objective : To evaluate the impact of short antiandrogen therapy on erectile function (EF) for localised prostate cancer using a validated patient-administered questionnaire (IIEF-5).

Design : Prospective non-randomised study with self-administered questionnaire.

Materials and Methods : A total of 90 patients, mean age 66 years (43-80), presenting with localised prostate cancer

and candidates for prostatic brachytherapy, received neoadjuvant antiandrogen therapy for up to 4.2 months (2-6). This consisted of LHRH agonist in 16%, non-steroidal antiandrogen (NSA) in 9%, cyproterone acetate in 41% and agonist + NSA in 34%. Erectile function (EF) before and after treatment was determined using the International Index of Erectile Function (IIEF-5) questionnaire. We asked the patients to describe the quality of their erection without PDE5 inhibitors. We obtained information regarding their erectile function before and after treatment in 58 of the 90 patients (64%). Patients who were not sexually active were excluded (n=4).

Results : Before treatment, 20 (37%) patients had no erectile dysfunction (ED) (score 22-25); 14 (26%) had mild (score 17-21), 13 (24%) mild to moderate (score 12-16), 3 (6%) moderate (score 8-11) and 4 (7%) had severe ED (score 1-7). Overall, 47 (87%) had a score above 12.

After treatment, 5 (12%) patients had no ED; 5 (12%) had mild, 16 (38%) mild to moderate, 5 (12%) moderate and 11 (26%) had severe ED. Overall, 26 (61%) had a score above 12.

Among the 47 patients whom initial score was above 12, after treatment 10 men (22%) had ceased to be sexually active, 15 (32%) deteriorated by two categories or more, 11 (23%) by one and only 11 men (23%) remained in the same category as before.

Conclusions : In most men who were previously sexually active, short antiandrogen treatment for prostate cancer had a deleterious effect on EF. One man in five ceased to be sexually active and one in three had a major deterioration of EF.

Support : None.

PO 094

Prognostic factors of erectile dysfunction in renal transplanted patients

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Objective : To determine the status of erectile function (EF) in men with renal insufficiency before and after renal transplantation, and prognostic factors of erectile dysfunction in this population.

Design : self-administrated questionnaire.

Materials and Methods : A series of 292 renal transplanted men were evaluated through a self-administrated questionnaire focusing on erectile function (IIEF5) regarding the period before transplantation (last month before) and after transplantation (at the date of the survey). Mean age was 53 +/- 12 years (23-79). Duration of hemodialysis before transplantation was 59 +/- 62 months (6 - 300). Duration of survey after transplantation was of 103 +/- 72 months (7 - 425). Immunosuppressant therapy consisted of cyclosporine in 120 patients, tacrolimus in 74 patients, sirolimus in 27 patients and a combination in the remaining cases. Questionnaire focused on EF without PDE5 inhibitors.

Results : Before transplantation, 125 (43%) patients had no erectile dysfunction (ED) (score 22-25); 56 (19%) had mild ED (score 17-21), 58 (20%) had mild to moderate ED (score 12-16), 19 (7%) had moderate ED (score 8-11), 31 (11%) severe ED (score 1-7) and 3 (1%) had no erection.

After transplantation, 134 (46%) patients had no erectile dysfuntion (ED) (score 22-25); 47 (16%) had mild ED (score 17-21), 48 (16%) had mild to moderate ED (score 12-16), 33 (11%) had moderate ED (score 8-11), 27 (9%) severe ED (score 1-7) and 3 (1%) had no erection.

EF was similar in the 2 groups treated by cyclosporine and tacrolimus (angioneurine inhibitors), and poorer in the sirolimus group (anti-proliferative agents) (p=0.01): Proportions of men having severe ED were 9.2%, 9.4% and 40.7% in the cyclosporine, tacrolimus and sirolimus, respectively.

EF was correlated with the duration of time elapsed from transplantation : The proportion of men having severe ED were 33.8% when the time after transplantation was less than 5 years and 9.4% when it was more than 5 years (p=0.02). EF was correlated with age (p=0.05), but not with the duration of hemodialysis treatment.

Conclusion : Prognostic factors of ED in renal transplanted patients are the age at transplantation(<50y), the time after transplantation(>5y), and the immunosuppressant therapy (angioneurin inhibitors >> antiproliferative agents).

PO 095

Erectile dysfunction in patients with myotonic dystrophy type 1 : correlation with sexual hormones

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Objective : Myotonic Dystrophy Type 1 (DM1) is an autosomal dominant disorder caused by a (CTG)n repeat expansion in

the DM1 protein kinase (DMPK) gene. DMPK gene expression is pleiotropic and includes premature development of age-related signs, symptoms and metabolic disturbances including testicular atrophy (63-82% of patients), hormonal dysfunctions and erectile dysfunction (ED). We evaluated the frequency and clinical characteristics of ED in DM1 and its relationship with sex hormone levels.

Design : We studied 32 consecutive DM1 men (age 22-60 yrs, median 41.5) with CTG expansion 60-1570 (median 287.5) and 32 age-matched healthy subjects.

Materials and Methods : All patients and control subjects were evaluated with an internationally validated 15-item questionnaire (International Index of Erectile Function-IIEF). In 20/32 patients (age 22-60, median 47.5) serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and testosterone (T) were measured by solid phase fluoroimmunometric assay (detection limits: 0.05 U/l for FSH; 0.05 U/l for LH; 0.04 ng/ml for PRL and 0.3 nmol/l for T). The reference ranges, calculated in 50 age-matched healthy subjects, were 1.6-8.2 U/l for FSH, 1.2-7.7 U/l for LH, 2.3-12.1 ng/ml for PRL and 9.4-33.5 nmol/l for T.

Results : 23/32 patients and 8/32 controls had ED ($p<0.001$). ED in DM1 was severe in 9, moderate in 4, and mild in 10 patients. Patients showed lower scores for erectile function ($p<0.001$), orgasmic function ($p=0.007$), satisfaction with intercourse ($p<0.001$) and overall satisfaction ($p=0.001$). In the control group, ED was moderate in 1 subject and mild in 7. Sexual desire was similar in both groups. CTG expansion was not correlated with severity of ED. Age, CTG expansion and ED severity were similar in patients who underwent sexual hormone assays (n=20) and those who did not (n=12). LH was increased in 6/20 patients, FSH in 11/20 and PRL in 2/20 patients and T was reduced in 2/20 patients. Patients with ED (n=15) showed higher levels of both FSH ($p=0.01$) and LH ($p<0.01$) than patients without ED (n=5). All patients with pathological values of both FSH and LH had ED. CTG expansion was directly correlated with both FSH and LH ($p<0.01$). Age was not correlated with sex hormone levels. 72% of patients in our DM1 series displayed symptoms of ED, though sexual desire was unaffected. Blood levels of FSH and LH were higher than normal in 55% and 30% of DM1 patients respectively. High levels of these hormones are correlated with ED.

Conclusion : These results demonstrate a strong correlation between DM1, hypergonadotropic hypogonadism and ED. Previous testicular damage may therefore be the main cause of the onset of ED, although other mechanisms such as neurological, metabolic and relational factors cannot be excluded.

Sexual rehabilitation with IC PGE1 injections and oral drug administration in diabetic patients non responder at oral therapy only

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Objective : Diabetes is an important risk factor in ED, acting via several mechanisms.

We assessed the efficacy of IC injections (ICI) rehabilitation and oral systematic therapy in diabetic patients, as well as the response of controls to oral therapy 'on demand'.

Materials and Methods : Sixteen diabetic patients with ED were treated with vasoactive drugs per os when needed without good results. The patients were then subjected to ICI rehabilitation with PGE1 20 mcg twice weekly for 4 weeks, followed by twice weekly administration of oral drugs for 4 weeks. Before and after rehabilitation, the patients completed a detailed anamnestic protocol to study their libido (always present) and they responded to questions Q3 and Q4 of the IIEF. During ICI, a study with ECCD was carried out.

All patients presented Type 2 diabetes: 10 were treated with oral antidiabetics, 4 were treated with insulin, and in the other 2 patients, treated with insulin, a sensitive neuropathy of the lower limbs was diagnosed. Fourteen patients were treated with antihypertensive drugs.

Results : Before rehabilitation, the mean responses to questions 3 and 4 of the IIEF were 1.6 and 1.5 respectively; after rehabilitation, the mean responses were 2.68 and 2.5 respectively.

The ECCD test showed an arterial component in 4 cases and a high EDV in 14 cases.

Four patients (25%) (2 with neuropathy and 2 with advanced age) did not respond to PGE1 or to oral therapy, 4 patients (25%) (2 treated with insulin and 2 oral) responded to ICI but not to oral therapy, while 8 patients (50%) showed a good response to both injectable and oral therapy, with good Q3 and Q4 scores.

Conclusions : Good endothelial function appears to be essential for the maintenance of acceptable erectile function. Diabetes has a negative effect on this function, as does hypoxia and low perfusion.

Based on the principle that a good erection improves endothelial function, we tried to determine if oral systematic

and intracavernous rehabilitation would improve erectile function in diabetic patients. The results indicate that diabetes interferes with erectile function, compromising the effects of the vasoactive drugs. However, integrated systematic rehabilitation appears to allow a good erectile response to both intracavernous and oral therapy in a large number of cases. Therefore, we suggest a rehabilitative protocol like this one in the treatment of ED in diabetic patients.

PO 097

Testosterone and metabolic syndrome : relationship with erectile dysfunction

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Objective : Assessing whether testosterone and metabolic syndrome (MS) are related to erectile dysfunction (ED).

Design : Prospective study in men consulting for ED in whom penile rigidity was measured.

Material and Methods : 153 men without local penile alterations consulting for ED were elected for study. Nocturnal erections with Rigiscan® were measured and rigidity under 60% as cut off point for ED was considered. NCEP ATPIII criteria was employed for diagnosing MS. Fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol, HDL Cholesterol, triglycerides, LDL Cholesterol. Total testosterone (TT), sex hormone binding globulin (SHBG), free testosterone calculated (TT x 100/SHBG) (FT). Blood pressure, body mass index (BMI) and waist circumference (WC) were taken. Univariate logistic regression models were established for each indicator.

Odds Ratios and its 95% Confidence Interval (CI95%) were displayed. Multivariate logistic regression models adjusted for age, BMI, Hb A1c, testosterone determinations and MS were established.

Results : The following tables gather the results of the study.

Conclusions : MS multiplies four times the risk for ED without taking into account testosterone. The risk of ED is higher with low FT in the presence of the MS. Elevated levels of Testosterone and FT combined with MS resist the individual effect over ED. When comparing groups TT determinations have no significant differences whereas FT seems to differentiate those with ED. Results in the present population indicate that ED is related to the metabolic syndrome and testosterone concentrations.

Table 1 : Comparison of characteristics according to ED.

	No Erectile Dysfunction (n = 96)	Erectile Dysfunction (n = 57)	P value
MS (percentage)	46 (49 %)	47 (80,7 %)	< 0,001†
Age (yr)	47,8 ± 8,9	57,2 ± 9,1	< 0,001
BMI (Kg/m²)	25,7 ± 4,3	28,7 ± 4,7	< 0,001
WC (cm)	99,7 ± 11,9	107,1 ± 11,4	< 0,001
FBG (mmol/l)	5,4 ± 1,2	6,8 ± 2,8	< 0,001
$HbA1_C$	5,8 ± 12,1	7,3 ± 2,3	< 0,001
TT (nmol/l)	19 ± 6,6	17,6 ± 7,4	0.2 (ns)
FT	0,6 ± 0,3	0,5 ± 0,2	0,004

Data represent means ± SD or percentages. NS : No Significant.

† : Chi-Square test

Table 2 : Odds Ratio in men developing ED without considering Testosterone levels

Significant results		Univariate Logistic Model Odds Ratio, ($CI_{95\%}$(OR))		
MS	No	1		
	Yes	4,36	[2,019	9,416]
Age		1,089	[1,049	1,13]
BMI		1,156	[1,068	1,252]
WC		1,055	[1,023	1,088]
FBG		1,566	[1,226	2,0]
HBA1c		1,907	[1,438	2,529]
By age categorizing				
Less than	50 years	1		
	51 to 60	3,240	[1,419	7,400]
		4,992	[2,104	11,841]

Table 3 : Multivariate logistic regression models.

Significant effects	Odds Ratio, [$CI_{95\%}$(OR)]		
Model 1 : Total Testosterone (TT)			
Age	1,094	[1,049	1,140]
BMI	1,131	[1,038	1,233]
MS by (TT) †			
No	1		
Yes	1,053	[1,012	1,095]
Model 2 : Free Testosterone (FT)			
Age	1,095	[1,051	1,141]
BMI	1,114	[1,021	1,216]
MS by FT†			
No	1		
Yes	3,893	[1,095	13,847]

† Interaction term

PO 098

Androtest : a structured interview for the screening of hypogonadism patients with sexual dysfunction

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Objectives : Detecting hypogonadism, important in general population, becomes crucial in patients with sexual dysfunctions, because hypogonadism can have a causal role for them and testosterone (T) substitution represents a milestone for the therapy. At present, three different inventories have been developed for screening of hypogonadism. All these instruments demonstrated a good sensitivity but low specificity. Furthermore, they were all developed for the screening of hypogonadism in the aging population, and not specifically designed for individuals with sexual dysfunction. Moreover, they are self-reported questionnaires (SRQ). This case-history tool has demonstrated important limits when compared to structured interviews (SI) in the evaluation of patients complaining for sexual dysfunction. Until now, there are no structured interviews available for the screening of hypogonadism in patients complaining for sexual dysfunction. Hence, aim of present study is the definition of a brief structured interview providing scores useful for detecting hypogonadism defined as low total T (< 10.4 nmol/L, 300 ng/dL) in a symptomatic population (sexual dysfunction).

Design : A minimum set of items was identified within a larger structured interview through iterative ROC curve analysis, with assessment of sensitivity and specificity for hypogonadism in a sample of 215 patients (Sample A).

Material and Methods : Sensitivity and specificity were verified in a further sample of 664 patients (Sample B). Correlation of test scores with PSA, testis volume, and others clinical and psychological parameters (Middlesex Hospital Questionnaire modified MHQ) was assessed for concurrent validity.

Results : The statistical analysis allowed the identification of a shorter interview format (12 items), including questions regarding age, history of delayed puberty, presence of pituitary diseases, history of cryptorchidism, presence of erectile dysfunction, reduced morning/nocturnal erections, frequency of autoerotism, feeling with autoerotism, presence of hypoactive sexual desire, reduction of the quantity of the volume of ejaculate, presence of mild/moderate delayed ejaculation, body mass index. The ROC curve analysis for the final version of the interview showed a sensitivity and specificity for hypogonadism of 76% and 66%, respectively (accuracy of 0.738±0.05; p<0.0001) when a threshold of > 8 was chosen. In the validation sample (Sample B), the final 12-item version of the interview (ANDROTEST) had a sensitivity and specificity of 68% and 65% (accuracy of 0.700±0.03; p<0.0001), in detecting hypogonadism and of 71% and 65% (accuracy of 0.716±0.03; p<0.0001), in the screening for low free T (<37 pmol/l). Furthermore, patients with pathological test (i.e score > 8) showed higher prevalence of hypogonadism related signs, such as lower testis volume (19.2±4.3 vs 20.1±3.8 ml, p<0.005) and higher depressive symptoms (MHQ-D score 6.1±3.1 vs 5.3±2.9, p<0.001). Finally, when younger patients only (<54 years, which represents the median age of the sample), were considered, Log10 [PSA] levels were significantly lower in those with ANDROTEST score >8 (-0.23±0.04 ng/ml vs –0.14±0.02 ng/ml, respectively ; p<0.05)

Conclusions : ANDROTEST is a quick, and easy-to-administer interview that provides scores for the screening of male hypogonadism in patients with sexual dysfunction. The determination of total and free T remains mandatory to confirm the diagnosis of hypogonadism.

PO 099

The metabolic syndrome and associated sexual dysfunction : psychobiological correlates

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Objectives : The aim of present study is to determine psychobiological characteristics of sexual dysfunction (SD) associated with metabolic syndrome (MS) as defined by National Cholesterol Education Program's Adult Treatment Panel III, (NCEP-ATPIII) criteria.

Design : A consecutive series of 803 patients attending for the first time to our University Outpatient Clinic for sexual dysfunction was studied.

Materials and Methods : Several hormonal, biochemical

and instrumental (penile doppler ultrasound, PDU) parameters were studied, along with psychopathology scores. The Structured Interview on Erectile DYsfunction (SIEDY), was also applied. This is a 13-item interview composed of three scales, which identify and quantify components concurring to sexual dysfunctions. Scale 1 deals with organic disorders, Scale 2 with disturbances in relationship with partner, and Scale 3 with psychological traits.

Results : Among subjects studied, 236 patients (29.4%) were diagnosed as having a MS. Among them 96.5% reported ED, 39.6% hypoactive sexual desire, 22.7% premature ejaculation and 4.8% delayed ejaculation. No significant difference, after adjustment for age, was observed in the prevalence of ejaculation disorders or in hypoactive sexual desire in patients with or without MS. Patients with MS were characterized by greater subjective (as assessed by SIEDY) and objective (as assessed by PDU) ED and by greater somatizated anxiety than the rest of the sample. In particular SIEDY scale 1 score (organic domain of ED), progressively increased as a function of number of MS components involved (B= 0.54±0.05; p<0.0001, after adjustment for age). Furthermore, basal and dynamic (after PGE-1 stimulation) peak systolic velocity at PDU showed a progressive decline as the numbers of MS components increased; this trend was significant even after adjustment for age (B= -0.79±0.23 and -2.23±0.69 cm/sec, respectively; both p<0.001). The prevalence of overt hypogonadism (total testosterone <8 nM) was significantly higher in patients with MS. Circulating total testosterone decreased as the number of MS components increased (B=-1.35±0.182 nmol/l ; p<0.0001, after adjustment for age). Accordingly, the relative risk for hypogonadism was significantly higher in patients reporting 3 or more risk factors for MS. Among MS components, waist circumference and hyperglycaemia were the best predictors of hypogonadism (OR=2.5[1.3-4.8] and 2.2[1.3-4.4] respectively). Hypogonadal patients with MS showed higher gonadotropins (5.8[3.5-11.9] vs 3.9[2.7-5.3] and 12.1[4.3-24.8] vs 5.1[3.4-8.3] U/l for LH and FSH respectively, both p<0.005) and lower free-testosterone levels, 24.2±11.9 vs 36.9±14 pmol/l, p<0.0001) suggesting a primary hypogonadism. Among patients with MS, hypogonadism was present in 11.9% and 3.8% in the rest of the sample (p<0.0001) and it was associated with typical hypogonadism-related symptoms, such as hypoactive sexual desire (66.7% vs 33.3% respectively in hypogonadal and non hypogonadal patients with MS, p<0.0001), low frequency of sexual intercourses and depressive symptoms.

Conclusions : Our data suggest that MS is associated with a more severe ED and induces somatization. Furthermore, MS is associated with a higher prevalence of hypogonadism in patients with SD. The presence of hypogonadism can further exacerbate the MS-associated sexual dysfunction, adding the typical hypogonadism-related symptoms.

A comparison of NCEP-ATPIII and IDF metabolic syndrome definitions with relation to metabolic syndrome associated sexual dysfunction

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Objectives : The aim of present study was to verify possible differences in the prevalence of vasculogenic erectile dysfunction (ED) and hypogonadism comparing two distinct new definitions of MetS, as National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII) and International diabetes Federation (IDF) in patients with sexual dysfunction.

Design : A consecutive series of 1086 patients attending for the first time to our University Outpatient Clinic for sexual dysfunction was studied.

Materials and Methods : Several hormonal, biochemical and instrumental (penile doppler ultrasound) parameters were studied. ANDROTEST Structured Interview was also applied. This a 12-item, recently validated, inventories, which assesses the degree of androgenization in male.

Results : The prevalence of metabolic syndrome was 32.0% and 44.7% according to NCEP-ATPIII and IDF criteria, respectively. Among patients with ED, MetS showed a significant correlation with PGE-1 stimulated penile flow (Vpmax) using both definitions (r=-0.187 and -0.123, for NCEP-ATPIII and IDF criteria, respectively ; all p<0.0001). At multivariate analysis, after adjustment for age, LDL-cholesterol and smoking status, only NCEP-ATPIII was significantly associated with Vpmax (B=-7.7±3.8; p<0.05). At logistic regression analysis, among NCEP-ATPIII components, hypertension, elevated glycaemia and hypertriglyceridaemia were significantly correlated with impaired Vpmax (< 30 cm/sec). Patients with MetS defined according to both criteria reported lower total (13.6±6.0 vs 17.4±7.2 nmol/l and 14.7±7.4 vs 18.2±6.0 nmol/l,) and free testosterone levels (34.8±14.0 vs 40.8±13.7 pmol/l and 36.2±14.1 vs 42.5±13.5 pmol/l), higher prevalence of hypogonadism (34.3 vs 11.9% and 25.3 vs 8.7%), and higher ANDROTEST score (9.6±3.0 vs 7.2±3.6 and 9.2±3.2 vs 6.0±3.2) respectively for NCEP-ATPIII and IDF ; all p<0.0001. To provide further information on possible

differences between definitions, multivariate regression analysis incorporating the five components of MetS were used to identify which component from each definition was independently associated with hypogonadism. These analyses identified that waist circumference, hyperglycemia and hypertriglyceridaemia were significantly associated with hypogonadism for all definitions. Incorporating into the same regression analysis both MetS criteria as putative predictors of hypogonadism, after adjustment for age and smoking habit, both NCEP-ATPIII and IDF were significantly associated with low testosterone (OR=2.55[1.03-6.33] and 1.62[1.05-2.50] for NCEP-ATPIII and IDF, respectively ; all $p<0.05$). In order to compare NCEP-ATPIII and IDF criteria for MetS, the prevalence of hypogonadism was assessed in patients fulfilling either one or both sets of criteria. When IDF, but not NCEP-ATPIII, criteria were fulfilled, the prevalence of hypogonadism was significantly lower than that observed in patients fulfilling both criteria (15.6 vs 34.8% respectively ; $p<0.0001$). Conversely, those fulfilling NCEP-ATP-III, but not IDF, criteria did not show a significant different prevalence of hypogonadism than those positive for both sets of criteria (30.8 vs 34.8% ; p=NS).

Conclusions : In patients with ED, NCEP-ATPII criteria seem to be a better predictor of hypogonadism and impaired penile blood flow than IDF.

PO 101

Assessment of the relational factor in male patients consulting for sexual dysfunction : the concept of couple sexual dysfunction

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Objectives : To date it is not clear to which extent a clinical, or even a subclinical, sexual dysfunction in the female partner might associate with erectile dysfunction (ED) in the male partner. The present study is aimed at the assessment of clinical features of ED associated with relational disturbances.

Design : A consecutive series of 1140 patients attending for the first time the Outpatient Clinic for ED sexual problems of the Andrology Unit of the University of Florence at Careggi Hospital and reporting a stable (more than 3 months) couple relationship was studied.

Methods : We evaluated the impact of relational factors, as assessed by SIEDY Scale 2 (exploring, as reported by the patient, menopausal symptoms, partner's medical illness interfering with sexual activity and reduced partner desire and climax). SIEDY is an easy to administer instrument for the first screening of ED patient, providing scores for the relational component (Scale 2) besides those to quantity the organic (Scale 1) and intrapsychic (Scale 3) components. Several hormonal, biochemical and instrumental parameters were also studied, along with psychopathology scores (Middlesex Hospital Questionnaire modified MHQ).

Results : The presence of a severe ED (inability to obtain an erection sufficient for intercourse in >75% of occasions) was significantly ($p<0.0001$) associated with Scale 1 ($r=0.376$) Scale 2 ($r=0.200$) and Scale 3 ($r=0.172$) score. The significant association between SIEDY Scale 2 and ED was confirmed at logistic multivariate analysis after adjustment for other SIEDY Scales and patient's and partner's age; in fact, the chance of being affected by severe ED increased by 10 [1-10] % for each increment of SIEDY Scale 2 score ($p<0.05$). Considering other sexual dysfunctions, we found that SIEDY Scale 2 score was higher in patients with mild to moderate delayed ejaculation (MMDE) when compared with patients without DE (2.97±0.51 vs. 1.97±0.07; $p<0.01$), while no difference was observed between patient with or without premature ejaculation. SIEDY Scale 2 scores are associated with an advanced age of the partner and a long couple relationship (>10 years), independently from patient's age. In addition, an increased relational factor significantly ($p<0.0001$) correlates with increased extra-marital affairs ($r=0.111$), conflicts in the couple ($r=0.279$), alcohol abuse ($r=0.155$) and presence of depressive symptoms ($r=0.182$), as assessed by MHQ questionnaire. The association between depressive symptoms and SIEDY Scale 2 was confirmed at multivariate regression analysis after adjustment for psychiatric diseases, partner's age, and the sum of MHQ score (Adj. r for MHQ-D= 0.253; $p<0.0001$). Furthermore, SIEDY Scale 2 score increases as function of the reduction in the frequency of sexual intercourses and in patients reporting a progressively severe hypoactive sexual desire even after adjustment for partner's age and depressive symptoms. Finally, SIEDY Scale 2 did not shown any correlation with pharmacological, biochemical, hormonal and instrumental parameters assessed.

Conclusions : Our result should encourage the andrologist to consider the context in which the sexual symptom develops, analysing the relationship and partner's behaviour and diseases. Resolving, or at least ameliorating, the relational background and the sexual framework might help in treating male sexual dysfunction.

PO 102

Psycho-biological correlates of free-floating anxiety symptoms in male patients with sexual dysfunctions

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Objectives : Anxiety has a relevant impact on everyday life, including sexual life, and therefore is considered the final common pathway by which social, psychological and biological stressors negatively affect sexual functioning. The aim of this study is to define the psycho-biological correlates of free-floating anxiety in a large sample of patients complaining erectile dysfunction (ED) based sexual problems.

Design : A consecutive series of 882 patients attending for the first time to our University Outpatient Clinic for sexual dysfunction was studied.

Material and Methods : Patients were interviewed prior to the beginning of any treatment, and before any specific diagnostic procedures, using the SIEDY Structured Interview. This is a 13 items structured interview, composed of three scales which identify and quantify organic, relational and intrapsychic domains. Metabolic and hormonal parameters, nocturnal penile tumescence test and penile doppler ultrasound (PDU) examination were also performed. MHQ-A scoring from Middlesex Hospital Questionnaire (MHQ) was used as putative marker of free-floating anxiety symptoms (AS). All statistical association among different parameters and MHQ-A scores were adjusted for total MHQ score, in order to discriminate the specific effect of free floating anxiety from generic psychological disturbances.

Results : At univariate analysis, MHQ-A score was significantly higher in patients complaining difficulties in maintaining erection in more than 25% of attempts when compared to rest of the sample (6.5±3.3 vs 5.8±3.3 ; $p<0.001$). When we considered other sexual dysfunctions, patients reporting hypoactive sexual desire and premature ejaculation showed significantly higher MHQ-A score when compared to the rest of the sample (6.9±3.3 vs 5.8±3.2 and 6.6±3.3 vs 6.1±3.3 respectively ; both $p<0.05$). No correlation was observed between SIEDY Scale 1 (organic component of ED) and free-floating anxiety symptoms. Conversely, MHQ-A score was significantly and positively related to both SIEDY Scale 2 (relational component of ED ; r= 0.101 ; $p<0.05$) and SIEDY Scale 3 scores (intrapsychic component of ED ; r= 0.323 ; $p<0.0001$). In particular considering relational factors, symptoms of free floating anxiety were significantly ($p<0.005$) correlated with the presence of couple's (r=0.111) or family's conflicts (r=0.141). Furthermore, a higher MHQ-A score was observed in patients reporting in their partner a low climax or low sexual desire when compared to the rest of the sample (6.5±3.2 vs 5.9±3.3 and 6.6±3.2 vs 5.9±3.3 respectively ; both $p<0.05$). Looking at intrapsychic factors MHQ-A score was higher in patients reporting low satisfaction and higher stress at work when compared to the rest of the sample (6.8±3.3 vs 5.4±3.2 and 6.8±3.4 vs 5.5±3.1 respectively ; both $p<0.0001$). Furthermore, symptoms of free floating anxiety were significantly ($p<0.05$) correlated with cigarette (r=0.108) and cannabis (r=0.072) smoking. Finally, among physical, biochemical or instrumental parameters tested, only end-diastolic velocity at PDU was significantly ($p<0.05$) higher in the 2nd versus the 1st quartile of MHQ-A (3[0-5] vs 0[-1-3.8] cm/sec; $p<0.05$), while the 3rd and 4th quartiles of MHQ-A did not show any further significant increase over the 2nd quartile.

Conclusions : In patients with ED based sexual problems, AS are correlated to many relational and life stressors. Conversely, organic problems are not necessarily associated with MHQ-A score.

PO 103

Psychological distress and erectile dysfunction

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Objective : The prevalence of mental disorders in men with erectile dysfunction (ED) is poorly known. Aim of this study was to investigate psychological distress in men with ED and to analyse whether mental disorders have a different prevalence in men with ED associated to atherosclerosis compared to men with ED of "presumed" non-organic origin.

Materials and Methods : The Symptom Check-List-90 (SCL-90), a validated multidimensional self-report questionnaire that scores nine primary symptom dimensions to identify psychological distress in outpatient psychiatric and non-psychiatric care settings, was applied to 250 men with ED (30 to 77 yrs). Intima-media thickness (IMT) of common carotid

arteries, estimated the degree of generalized atherosclerosis by B-mode ultrasound, and pharmacologically stimulated peak systolic velocity in cavernous arteries estimated cavernosal perfusion disorders.

Results : 34% of patients showed psychological distress as defined as the presence of at least 1 of 9-subscale index higher than 90th percentile. Depression and anxiety were respectively observed in 8.8% and in 9.6% of cases. Body mass index (BMI) positively correlated with each of 9 dimensions, while a negative correlation was found between the severity of ED (SHIM test) and anxiety (p=0.010) even after controlling for BMI. No differences were found between men with or without vascular risk factors in the mean value of the 9 subscale scores although men with atherosclerosis (IMT≥0.09 mm) showed an increased number of cases with scores >90th percentiles in all subscales ; in particular atherosclerosis was associated to an increased risk of obsessive-compulsive disorders (OR 3.18, CL 1.32 to 7.61) after controlling for age, BMI, testosterone level.

Conclusion : One third of patients with ED showed psychological distress. This seemed to be more prevalent in men with vasculogenic ED thus confirming the association between clinical atherosclerosis and mental disorders. At variance with cardiac and cerebral vascular disease, vasculogenic ED showed an increased prevalence of obsessive-compulsive disorders, which relevance for the efficacy of pharmacologic control of ED needs to be determined.

PO 104

Psycho-biological correlates of delayed ejaculation in male patients with sexual dysfunctions

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Objectives : Pathogenesis of delayed ejaculation (DE) is rather unknown, although the contribution of various psychological, marital, hormonal and neurological factors has been advocated. The aim of the present study is to investigate on the psychobiological factors associated with DE in a large sample of patients referring to an Andrology Clinic for sexual dysfunction.

Design : We systematically investigated the relative relevance of the aforementioned factors in 1632 men, seeking medical help for sexual dysfunction.

Material and Methods : Patients were interviewed prior to the beginning of any treatment, and before any specific diagnostic procedures, using the SIEDY Structured Interview. This is a 13-item interview composed of three scales, which identify and quantify components concurring to sexual dysfunctions. Scale 1 deals with organic disorders, Scale 2 with disturbances in relationship with partner, and Scale 3 with psychological traits. The severity of DE was classified according to Kaplan criteria on a 3-point scale using a standard question "In the last three months is it difficult to ejaculate during sexual intercourse?" and rating : 0 (no DE) ; 1 (mild/moderate DE or MMDE) and 2 (anejaculation/severe DE or ASDE). MMDE was diagnosed if ejaculation and climax were still possible, but only with great effort and after prolonged intercourse (mild DE) or possible only with autoerotism, although in the presence of the partner, but not during coitus (moderate DE). ASDE was diagnosed if orgasm and ejaculation could not be obtained at all (anejaculation) or could be obtained but only with autoerotism conducted in the absence of the partner (severe DE).

Results : Among the 1632 patients studied, 82 (5.0%) reported DE ; of those, 62 reported MMDE and 20 ASDE. Mild and moderate forms of DE (MMDE) recognized different risk factors than the most severe ones (anejaculation/severe DE; ASDE). ASDE was essentially coupled to the presence of neurological diseases (OR= 10.1[4.1-24.8]) or to the use of serotoninergic drugs (OR=4.1[1.2-14.7]). Serotoninergic drugs also significantly increase (OR=11.0[5.8-21.2]) the risk for MMDE, which, however was also coupled to other relational (impaired partner's climax, patient's hypoactive sexual desire, HSD ; OR=2.7[1.4-5.0], 3.4[2.0-5.7] respectively) or intra-psychic (stress at work, OR=1.9[1.9-9.5]) factors. At multiple regression analysis, some organic pathological conditions (such as psychiatric disorders and hypogonadism) were also associated to MMDE (OR=3.3[1.7-6.6] and 2.4[1.3-4.4] respectively). In particular, hypogonadism (total testosterone < 10.4 nmol/l) retained significance for DE even after adjustment for HSD (Adj. OR= 2.08[1.11-3.89] ; p<0.05), suggesting other effects of testosterone deficiency on the ejaculatory reflex, besides reduced libido. Finally, patients with MMDE showed higher SIEDY scale 2 and 3 scores when compared with the rest of the sample (2.8±0.5 vs 1.9±0.1 for scale 2 and, 5.9±0.3 vs 5.2±0.1 for scale 3, both p<0.05) while no difference was observed in scale 1 score (4.2±0.4 vs 3.7±0.1 p=NS). On the other hand, patients with ASDE did not show any significant difference in SIEDY scores in comparison with the rest of the sample.

Conclusions : The present study demonstrates that multiple psychobiological determinants are associated to DE, a still obscure condition that substantially impairs psychosexual equilibrium of the couple.

Sildenafil citrate and clomipramine versus clomipramine alone in the treatment of premature ejaculation

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Objective : Premature ejaculation (PE) is the most common ejaculatory dysfunction. We compared the efficacy of sildenafil citrate and clomipramine combination with clomipramine alone to increase the time to ejaculation, and improvement of partner satisfaction.

Design : A randomized open-label clinical trial.

Materials and Methods : A total of 80 patients with erectile dysfunction were treated in a randomized, parallel-group study conducted at outpatient clinic of Shohada-e-Ashayer hospital in Khorram-Abad city of Lorestan province, the west of Iran. Forty patients received clomipramine 25mg/po/day [group A]. The latter group received 25 mg of oral clomipramine per day, and 50 mg of oral sildenafile 60 minutes before each intercourse [group B]. Each patient received drugs for 3 months. Intravaginal ejaculatory latency time (IVELT) and rate of partners sexual satisfaction (scored: 0-10) were recorded at the beginning and the end of the study.

Results : Mean IVELT at the beginning of the study was 2 ±1.1 minutes and 2.6 ± 1.55 minutes in groups A and B respectively (P=ns). After 3 months mean IVELT increased to 3.42 ± 1.8 minutes, and 5.45± 1.4 in groups A and B respectively. IVELT increasing was significantly higher for those receiving sildenafile and clomipramine combination (P=0.001). Partners of 8 patients in group A (20%), and 11 patients in group B (27.5%) satisfied of their intercourse at the beginning (P=ns). At the end of the study, this rate changed to 42.5% (17 partners) and 92.5 %(37 partners) respectively (P=0.001).

Conclusions : Sildenafil in combination with clomipramine is more effective than clomipramine alone in patients with premature ejaculation.

First report on ejaculation after brachytherapy for localised proatate cancer

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Objective : To evaluate ejaculation before and after (125)I brachytherapy for localized prostate cancer using a self-administered questionnaire.

Design : Prospective non-randomised study with self-administered questionnaire.

Materials and Methods : A total of 316 patients underwent permanent implantation of (125)I seeds between July 2000 and May 2006 for localised prostate cancer. No patient received supplemental external beam radiation therapy ; 29% received adjuvant antiandrogen therapy for up to 4 months. Of the 316 patients, 210 answered the questions about erection and 198 (63%) completed the items concerning ejaculation. Fourteen patients who were not sexually active were excluded. Mean age was 66 years (43-80). Mean follow-up was 4 years (2-6). The questionnaire focused on erectile function and ejaculation.

Results : Before treatment, 172 (87%) had no or mild erectile dysfunction and 179 (90%) had ejaculation. Nine patients described anomalies of ejaculation: pain in 1 case, haemospermia in 2, light or fluid semen in 2, clotted semen in 4 cases.

After treatment, 93 (55%) had no or mild erectile dysfunction and 138 (70%) conserved ejaculation. However, 120 (87%) reported a decrease in semen volume. Thirty-four patients described anomalies of ejaculation : pain in 13 cases (9%), haemospermia in 12 (9%), light or fluid semen in 5 (4%), clotted semen in 4 cases (3%). Median time to appearance of anomalies of ejaculation was 4 months (2-12).

Conclusions : This study is the first to focus on ejaculation after prostate brachytherapy. It reveals that ejaculation is conserved in more than two-thirds of patients, even if they described changes of volume and consistency.

Support : None.

PO 107

Altered endocytotic pathways of epidermal growth factor receptor in androgen receptor positive prostate cancer cell lines

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Although androgens and the androgen receptor (AR) are involved in tumorigenesis of prostate cancer (PC) in initial phases, less clear is their role in androgen-independent disease. Recent reports indicate that re-expression of AR in PC cell lines determines a less aggressive phenotype of the cells. We have observed that re-expression of AR in the androgen-independent PC cell line PC3 decreases invasion of PC3-AR cells in vitro by interfering with signalling and internalization of EGF receptor (EGFR).

Here we show that a reduced EGFR internalization (evaluated both by immunofluorescence and flow cytometry techniques) is also characteristic of AR positive PC cell lines LNCaP and 22Rv1. Our data demonstrate that the reduced EGFR internalization in PC3-AR cells is due to an alteration of the ability of the receptor to interact with 2 adaptor proteins that play a key role in EGFR endocytotic signalling, namely Grb2 and c-Cbl. Indeed, by immunoprecipitation studies we found that Grb2 and c-Cbl association with EGFR were highly reduced in PC3-AR cells respect to the parental cell line. As consequence of such reduced interaction, ubiquitination of the receptor, which is mediated by the ubiquitin ligase c-Cbl, was also found altered in PC3-AR cells.

In addition, we report evidence that expression of AR determines a shift of internalization pathway from the clathrin-coated pit one (which support full signalling and recycling of the receptor) to the raft-mediated one (which is mainly involved in lysosomal degradation of the receptor). Indeed, while following internalization EGFR clearly co-localizes with early endosome antigen-1, a marker of clathrin-coated pit pathway, in PC3-Neo cells, such a co-localization was not observed in PC3-AR or the other AR positive PC cell lines. Conversely, when caveolin-1, a marker of raft-mediated endocytosis, was studied, EGFR maintains co-localization with caveolin-1 after EGF in PC3-AR cells but not in PC3-Neo.

We suggest that AR expression affects EGFR clathrin-mediated endocytosis pathway, which plays a key role in the signalling of the receptor, as many signalling pathways are activated only when the receptor is internalized through this route. These data highlight the role of AR in regulation of EGFR endocytosis and signalling in PC cells. In view of the role of EGFR signalling in invasion of PC cells, our data may explain the lower invasive phenotype observed in AR-positive cell lines.

PO 107 bis

Study of gene espression of recurrent fusion between the androgen-responsive gene TMPRSS2 and ets transcription factor erg in prostate cancer

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Prostate cancer (PC) is the second most commonly diagnosed malignancy in American men and displays different clinical behaviours from indolent to metastatic disease. It is not clear which molecular events are responsible for the progression of PC to a lethal form. Genes involved in carcinogenesis have often been identified through analysis of recurrent chromosomal rearrangements but they have not been identified in carcinomas until recently, when Tomlins et al (2005) applied bioinformatics techniques to identify oncogenic chromosomal changes based on analysis of outlier gene expression.

The authors determined that two ETS transcription factors, ERG and ETV1, were outliers in PC. They show recurrent fusions of the 5' untranslated region of the androgen-dependent TMPRSS2 gene to ERG and ETV1 genes in the majority of PC samples containing the outlier expression. We evaluated the occurrence of the chromosomal rearrangement by profiling gene expression of the above mentioned translocation on chromosome 21 in 50 primary PC samples as well as in the surrounding normal prostate tissue. To identify TMPRSS2:ERG and TMPRSS2:ETV1 fusions we performed both standard Reverse-transcription PCR (RT-PCR) followed by electrophoresis of the products and quantitative PCR (QPCR) by using SYBR Green dye on a Real Time PCR system. Freshly frozen prostate surgical specimens were obtained through radical prostatectomy from Urological Surgery in the years 1998-2000 and carcinoma tissue was identified, excised and immediately frozen in liquid nitrogen. Clinical data and follow-up of patients are available for most of the subjects.

Our data confirm the high percentage of expression of the fusion gene in PC. In particular, we found a percentage of translocation expression of 70% in our cohort of patients. The translocation was completely absent in the surrounding normal tissue. In several samples we obtained a pattern of expression characterized by bands with different molecular weight. In some PC samples the expected band with appropriate molecular weight was sequenced. Sequence analysis confirmed the fusion of the complete exon 1 of TMPRSS2 with the beginning of exon2 of ERG. No translocations were found for the ETV gene in our cohort of patients.

Our data confirm that this fusion gene product may play a key role in the development, diagnosis, and treatment of PC.

PO 108

Relationship between non-enzymic antioxidant profile and mean prostate specific antigen (mPSA) levels of known prosate cancer patients

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Objective : Oxidative stress has been implicated in the etiology of several pathologies, prostate enlargement inclusive. The present study was designed to relate the non-enzymic antioxidant levels in prostate cancer patients with their mean prostate-specific antigen (mPSA) values.

Patients and Methods : Participants were recruited (with informed consent) from the Cancer Screening Unit (CSU), University College Hospital (UCH), Ibadan, Nigeria. 120 prostate cancer patients were assigned into 3 groups on the basis of mPSA values; group 1 with mPSA of 6.5 µg/L, group 2 with mPSA of 15.9 µg/L and group 3 with mPSA of 73.8 µg/L. Patients had no recent hormone therapy and/or radiation therapy. Likewise, 120 apparently normal subjects were recruited as control and had mPSA value of 2.8 µg/L. The study was approved by Ethical Committees of the UCH and Oyo State Government of Nigeria.

Results : Patients with mPSA µ 6.5µg/L to 73.8 µg/L had significantly lower serum uric acid and vitamin E levels ($p < 0.001$) than the control. Significant reduction ($p < 0.001$) in serum vitamin C levels were also observed in patients with mPSA of 15.9 µg/L to 73.8 µg/L when compared to the control. Precisely, serum vitamin C levels were decreased by 32% and 47% in patients with mPSA of 15.9 µg/L and 73.8 µg/L, respectively. The extent of lipid peroxidation (LPO) in the sera of patients was estimated by measuring the thiobarbituric acid reacting substances (TBARs) formed. Serum LPO was significantly elevated ($p < 0.001$) in patients with mPSA of 6.5 µg/L to 73.8 µg/L when compared to the control. Specifically, LPO was elevated by 28%, 35% and 46% in patients with mPSA of 6.5, 15.9 and 73.8 µg/L, respectively. Furthermore, serum selenium levels were decreased by 35%, 34% and 38% in patients with mPSA of 6.5, 15.9 and 73.8 µg/L, respectively.

Conclusions : These results indicate an inverse relationship between the non-enzymic antioxidant profile of prostate cancer patients and their respective mPSA values. This relationship should not be overlooked and will serve as basis to further understand the biochemical aspect of the disease.

Support : Self-sponsored study.

PO 109

An androgen-dependent PDE5 activity in bladder might contribute to luts

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Objective : BPH is the most common disease in male aging, often associated to Erectile Dysfunction (ED). PDE5 inhibitors (PDE5i) ameliorates LUTS in patient with ED and BPH. We therefore studied their action in the rat and human bladder.

Design and method : We studied PDE5 expression and activity in human bladder and effects of PDE5i using in vitro (human and rat) and in vivo (rat bladder outlet obstruction, BOO) models.

Results : In bladder tissues, we found high PDE5 mRNA expression and an intense immunolocalization of PDE5 protein in the vascular endothelium and smooth muscle cells. PDE5i

vardenafil, sildenafil and tadalafil, blocked 70% of the total cGMP catabolizing activity in human bladder homogenates. Vardenafil exhibited the highest IC_{50} (0.3 nM). In male rat, the NO-donor sodium nitroprusside (SNP) only marginally relaxed carbachol-precontracted bladder strips, while its activity was strongly potentiated by vardenafil (100 nM) to the level of the PDE-resistant cGMP analogue SP-8-Br-PET-cGMPS. According to these results, in human bladder stromal cells the anti-proliferative activity of SNP was strongly potentiated by vardenafil up to the PDE-resistant cGMP analogue. We also found that surgical castration in the rat decreased, and T supplementation restored, PDE5 gene expression (Real-time RT-PCR) and enzymatic activity. Accordingly, bladder strips from castrated rats were more sensitive to SNP induced-relaxation than strips from control or T-replaced rats, while in presence of vardenafil, all the groups showed the same SNP sensitivity. To elucidate whether vardenafil affects in vivo bladder activity, the rat BOO model was used. Chronic treatment with 10mg/kg/d vardenafil did not influence bladder hypertrophy but significantly reduced (47%, $p<0.05$ vs placebo) non-voiding contractions in the rat to the level of tamsulosin (51%).

Conclusions : Overall, these results demonstrate that PDE5 in the bladder regulates smooth muscle tone, strongly limiting the NO/cGMP signalling, and that vardenafil, by blocking PDE5, might represent a possible therapeutic option for bladder dysfunction.

PO 110

BXL-628, a vitamin D analog, decreases RHO/ROK signalling in rat and human bladder

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Objectives : BXL-628 is a non-hypercalcemic calcitriol analog successfully tested in a phase IIa trial for benign prostate hyperplasia therapy. Because part of low urinary tract symptoms (LUTS) are generated by overactive bladder (OAB) and bladder expresses the calcitriol receptor (VDR), we investigated the BXL-628 effects on bladder contractility and RhoA/ROK signalling activated in OAB. Design and Methods : In vivo (2weeks; 30 mcg/Kg, Sprague-Dawley, SD, and spontaneously hypertensive rats, SHR) and in vitro (human bladder stromal cells, hBC) experiments were carried out to investigate the BXL-628 effect.

Results : In SD bladder, BXL-628 did not affect maximal responsiveness to carbachol but increased the lag time to reach it, and reduced the maximal relaxant effect of the ROK inhibitor Y-27632. In SHR bladders, that over-express RhoA/ROK pathway and develop OAB, the Y-27632 effect was higher than in normotensive control rats Wistar-Kyoto (WKY). BXL-628 normalized SHR bladder sensitivity to Y-27632, while did not significantly affect RhoA/ROK expression (qRT-PCR) in rat bladder and in hBC. In immunokinase assay BXL-628 significantly decreased ROK activity, by reducing Y-27632 effect, of hBC extracts immunoprecipitated with anti-ROK. In SHR bladders ROK activity was higher than in WKY and significantly reduced by BXL-628. We therefore investigated whether BXL-628 impaired RhoA activation in hBC. Confocal microscopy, using pancadherin (a membrane marker) and RhoA immuno-labelling, revealed a reduction of RhoA membrane expression, which indicates its activation state, in BXL-628 treated hBC. Accordingly, BXL-628 significantly reduced the activated, rhotekin-bound, RhoA fraction in hBC (Western blot). Because RhoA is involved in cell motility, we tested the effect of BXL-628 on hBC migration. BXL-628 even at 0.01pM inhibited cell migration as other inhibitors of RhoA (simvastatin, C3 exoenzyme). Calcitriol was effective at higher concentrations (1nM).

Conclusions : in human and rat bladders, BXL-628 inhibits RhoA/ROK signalling, suggesting a novel therapeutic opportunity for OAB and LUTS treatment.

PO 111

The comparison of the three lobes of the rat prostate in aging process and in hyperprolactinemia induced by haloperidol

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Objective : The aim of the conducted studies was to establish the influence of hyperprolactinemia on epithelium and stroma

in the three lobes of the rat prostate. We compared changes in morphology and selected proteins expression in each lobe of the rat prostate.

Design : Sexually mature 18-month-old male Wistar rats were randomly divided into two groups: experimental and control ones. Each of the groups consisted of eight animals. The rats in the experimental group were intraperitoneally administered haloperidol (HAL) in a dose of 2.0 mg/kg body mass for 14 days.

Materials and methods : Immunoezyme method was used for the measurements of hormones serum concentrations: prolactin (PRL) (Spi-Bio, France), testosterone (T) (AxSYM Testosterone test MEIA, USA) and estradiol (E) (DPC Immulite 2000, France). For light microscopic examinations, the slides of each lobe of the rat prostate were routinely obtained, prepared and stained with standard H-E and p.a.S. method. Immunohistochemistry standard ABC techniques were used to compare expression of prolactin receptor (PRLR) (Affinity BioReagents, USA), androgen receptor (AR) (Novocastra Lab, UK) and estrogen receptor β (ERβ) (Affinity BioReagents, USA). To compare expression of proliferating cell nuclear antigen (PCNA) and desmin and vimentin filaments, modified immunohistochemical techniques were used with DAKO EnVision System HRP (Dako/AS, Denmark).

Results : The rats in the experimental group (administered HAL) showed about 2 times higher the mean PRL concentration (50.23 ± 14.15ng/ml vs 24.53 ± 4.07ng/ml) and increased E (30.38 ±11.30 pg/ml vs 21.02 ±2.28pg/ml), whereas the mean concentration of T (0.46 ± 0.32 ng/ml vs 0.96 ± 0.31 ng/ml) was decreased in comparison to the mean concentration in control group. Changes in morphology affected each lobe of the prostate. Lateral and dorsal lobes revealed hypertrophy of glandular ducts with dysplasia markers especially in lateral lobe. Focal hyperplasia in epithelium suggested digitate outgrowths and multistratified cells clusters, and was confirmed with raised PCNA expression. In the stroma of these two lobes, desmin concentrated surrounding glandular ducts and forming thick sheath especially in dorsal lobe. Ventral lobe, due to reduced T concentration, revealed atrophic features – reduced proliferate activities and smoothening of the glands surface. In the stroma there were noticed inflammatory infiltrations with focal fibromatosis, and strong expression of desmin and vimentin filaments especially in dorsal and lateral lobes as well as changes in the ultrastructure of the columnar epithelial cells. Immunohistochemical study revealed expression of AR with ERβ in the nuclei and expression of PRLR in cytoplasm of columnar epithelial cells in each lobe of the rat prostate in both experimental and control groups.

Conclusion : We postulate that prolactin in male rats, especially in overnormal concentrations, affects the prostate epithelium and stroma morphology as well as expression of AR, ERβ and PRLR individually in each lobe of the prostate.

A evidence based approach to the diagnosis of late - onset hypogonadism (LOH) in men using data from the european male ageing study (EMAS)

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Prevalence of 'hypogonadism' of ageing has been reported at between 20-50% in 60-90 year old men using biochemical thresholds alone. However, recent guidelines recommend combining clinical features suggestive of androgen deficiency together with low total testosterone (TT) to furnish a more rational basis for diagnosis. However, there is no consensus as to the nature, number or severity of symptoms to identify these cases of 'hypogonadsim' in the ageing male population.

The present study aims to identify the most informative clinical symptoms associated with testosterone deficiency. Our analyses were based on data from The European Male Ageing Study (EMAS), a population-based cohort study of men aged 40 to 79 years from 8 European cities (Manchester in the United Kingdom, Malmo in Sweden, Tartu in Estonia, Szeged in Hungary, Lodz in Poland, Leuven in Belgium, Florence in Italy and Santiago in Spain). All 3369 men completed self-reported symptom questionnaires and had morning total T (TT) and sex hormone binding globulin (SHBG) measured by immunoassay, (Roche, Elecys E170) from which free T (FT) was calculated. From a total of 31 possible symptoms potentially related to T deficiency, only 5 (2 sexual and 3 physical symptoms) were independently associated with TT and FT levels.

Using the relationships between these 5 symptoms with TT and FT, we employed a statistical technique of piecewise logistic regression and nonparametric regression LOWESS (The LOcally WEighted Scatter plot Smoothing) to identify testosterone thresholds below which a significant increase in risk of developing a deficiency symptom was observed. This revealed clear threshold levels of TT for the sexual symptoms but thresholds were not as well-defined for physical symptoms. FT levels showed less clearcut relationships with symptoms.

Our results highlight different dose-response relationships between specific symptoms with TT and/or FT. Nevertheless, we demonstrate that an evidence-based, clinically-pertinent approach can be applied to establish objective criteria on which a rational diagnosis of hypogonadism can be made in symptomatic middle-aged and elderly men. These results also suggest that the true prevalence of testosterone deficiency may be considerably lower than previously estimated.

PO 113

What is the Association between Testosterone and Frailty in Ageing Men ?

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Introduction : Frailty is a common cause of disability and dependency in the elderly. The aetiology of frailty is multifactorial. However, ageing-associated endocrine dysregulation may play an important role. It is not known if low testosterone (T) contributes to frailty, through its effects on muscle (sarcopenia) and physical function (strength and endurance).

Objective : To investigate the relationship between T and frailty in elderly men by examining the associations between T and various degrees and components of frailty.

Design : Cross-sectional observational study in community-dwelling men, who responded to invitation letters to participate in a clinical trial of testosterone replacement therapy.

Methods : One thousand and thirty one, men, median age 72 yr, (range 65-95) were screened for the presence of frailty using Fried's criteria1 (weight loss of 10 lbs or more in the past year, self-reported exhaustion, decreased grip strength, slow walking speed and reduced physical activity). Frail (F) men were characterized by the presence of 3 criteria, prefrail (PF) men by 1-2 criteria and non-frail (NF) men by the absence of any criteria. Serum samples were obtained before 11.00 a.m for the measurement of total testosterone (TT), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), and luteinizing hormone (LH). Free testosterone (fT) was calculated using the Verumulen formula.

Results : The prevalence of F and PF was 9% and 45 % respectively. Mean (95%CI) TT was lower in F and PF; 12.2 (11.1,13.4) and 13.9 (13.3,14.4) nmol/L respectively vs. 14.3 (13.7,14.9) nmol/L for NF (age adjusted p value, 0.037). Mean fT was also lower in F and PF; 0.18 (0.17,0.2) and 0.22 (0.21,0.22) nmol/L respectively vs. 0.23 (0.22,0.24) for NF (age adjusted p value, <0.001). F and PF men also had higher levels of FSH and LH (age adjusted p values, 0.001 and 0.029 respectively) compared to NF. An inverse relationship was observed between TT, fT and the number of frailty components. In a multinomial logistic regression analysis at a TT of <12 nmol/L, the age adjusted odds ratio (OR) (95% CI) for being F was 1.77 (1.07, 2.95) (P = 0.02) when compared to being NF. The presence of walk time criteria was significantly associated with a TT <12 nmol/L with an OR (95% CI) of 2.9 (1.6,5.3) (P <0.001). Weight loss criteria was significantly associated with a TT of <10 nmol/L with an OR (95% CI) of 1.7 (1.01, 2.9) (P=0.04).

Conclusions : Low T levels are associated with frailty and some of its components (walk time and weight loss). However, the role of testosterone needs to be assessed longitudinally before a causal relationship can be attributed to testosterone in the aetiology of frailty.

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Support : Schering AG, Berlin.

PO 114

Testosterone Levels Among Octogenarians

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Background : Circulating testosterone (T) levels decline gradually with increasing age in men. However, the age-related decline in several functional domains (e.g. frailty, cognitive deficits, osteoporosis) accelerates in the eighth decade of life. It is not known if the loss of function in the oldest old is related to a more precipitous fall in T due to the relative paucity of hormonal data in extreme old age.

Objective : This study investigates the hypothesis that total

testosterone (TT) and free testosterone (FT) levels decline more rapidly in men in the eighth compared to those in the sixth and seventh decade. We also sought to determine, if factors influencing T levels among octogenarians are different from earlier decades.

Design : Cross-sectional observational study in community-dwelling men, who responded to invitation letters to participate in a clinical trial of testosterone replacement therapy for frailty.

Methods : One thousand three hundred and twenty men, mean age, 73 (range 65-95) years were included in this study. Medical, drug and smoking histories were obtained by self-report. Height and weights were measured and body mass index (BMI) calculated. Serum samples were obtained before 11.00 a.m. for the measurement of total testosterone (TT), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), and luteinizing hormone (LH) (Roche. Elecys E170). Free testosterone (FT) was calculated using the Verumulen formula.

Results : The entire cohort was divided into 4 age bands: B1 (65-69 years, n =510), B2 (70-74 years, n=357), B3 (75-79 years, n=258) and B4 (80-95 years, n=195). There was no significant difference in TT in B4, compared to other age bands (13.4 nmol/L vs.13.9, 13.7, 13.9 nmol/L for B1, B2 and B3 respectively, p=0.63). Over 60% of men in B4 had a TT >12 nmol/L, not significantly different from other age bands. FT was significantly lower in B4, than in other age bands (0.18 nmol/L vs. 0.23, 0.21, 0.20 nmol/L for B1, B2 and B3 respectively, p <0.001). The fall in FT with age was linear; there was no rapid decline in FT in B4. B4 had significantly higher SHBG, LH and FSH when compared to other age bands. There was no significant difference in the prevalence of various comorbidities among age bands except cerebrovascular disease, which was higher in B4 (p=0.01). There was also no difference in the number of comorbidities among various age bands (mean [SD] 1.53 [1.4], 1.74 [1.40], 1.81[1.4], 1.66 [1.4], [P=0.051] for B1, B2, B3 and B4 respectively). Men in B4 were more likely to be exsmokers (p<0.05) and less likely to be current smokers p<0.01). Age, BMI, chronic obstructive airways disease and cancer were independently associated with TT (ß = - 0.06, -0.44, - 1.14 and -2.46 respectively) and FT (ß = –0.003, -0.004 and -.018 respectively) in the entire cohort. Increasing number of comorbidities were also associated with falling TT levels (ß = -1.16, -1.17 and –1.80 for 2, 3 and >4 comorbidities respectively). A similar trend was found for FT, but only in men with 3 or more comorbidities. Analysis of the four individual age bands revealed that BMI was associated with TT in all age groups. Chronic obstructive airways disease was associated with falling TT only in B2 (ß= –1.72, p= 0.04). Cancer was associated with TT (ß = –4.82 and –4.53) and FT (ß = –0.72 and –0.54) for B3 and B4 respectively. In all age bands BMI was associated with FT, except B4, where age, not BMI was significant (ß = –0.005).

Conclusions : TT does not fall at a faster rate in men in the eighth decade and older. Factors associated with lower TT and FT do not differ in octogenarians compared to younger men.

Support : Schering AG, Berlin.

Post- vasectomy complications in Khorramabad city, the west of Iran

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Objective : Vasectomy as a permanent form of birth control is the safest and easiest form of surgical sterilization, but studies on complications which may arise from this procedure are not enough reproduced. In this study we assessed the frequency of some late complications of vasectomy in a sample of Iranian patients.

Design : A cross sectional study.

Materials and Methods : At August 2004, invitation letter were posted to 324 men whom had underwent vasectomy in the health service center number III of Khorram-Abad city, in western Iran between March 1998 and September 2002. We asked them to come to urology clinic of the University for Follow-up and checkup. Data including their libido, impotency, ejaculation disorders, psycho-somatic and sleep disorders, chronic orchalgia, granuloma formation, joint pain, procedure failure, and surgical regretting were gathered.

Results : Within 6 months, 110 of invited men came to the clinic. 40% of the patients had libido variations. 14.5% reported impotency which occurred after the procedure. 10.9% had premature ejaculation. Mood and sleep disorders were detected in 10% and 4.5% of the patients respectively. 20%, 14.5%, and 9.8% of the patients showed chronic orchalgia, granuloma formation, and joint pain respectively. 2.7% of the patients regret vasectomy. There was no failure after clearance in operated patients.

Conclusions : In comparison with studies from other regions, chronic orchalgia and psychiatric problems were more common in our patients. Whether it relates to socio-culture status or vasectomy procedure needs to be assessed.

Female Genital Mutilations and sexual troubles

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Objectives : The goal of this study was to describe female genital mutilations and to assess their impact in sexual life among a population of non selected women.

Patients and Methods : We underwent a prospective study among a population of women randomly selected. All these women filled a questionnaire. Therefore we realized a case-control study including 176 women victims of female genital mutilations whom we compared to 352 women without a history of genital mutilation.

We excluded of this study pregnant women, not healthy women and women younger than 18 years.

The data studied were: sexual desire, sexual arousal, sexual pleasure, dyspareunia and sexual satisfaction.

For the statistical analysis, we underwent simple and cross tabulations. Chi square was used for data comparison. p was significant when less than 0.01.

Results : In our study population, 71.8% of circumcised women experienced sexual dysfunctions compared to 56.4% of non circumcised women. Excision was found to increase the risk of luck of sexual desire 2.9 fold while infibulation increased the same risk 8.2 fold. Excision increases the risk of feeling no pleasure 3.2 fold.

Conclusion : Female genital mutilations represent a significant risk factor of developing sexual dysfunctions. More studies should be carried out to better assess the negative impact of that practice.

Keywords : Female genital mutilations, sexual dysfunctions, cultural practice.