ANDROLOGY (MALE FERTILITY, SPERMATOGENESIS)

P-001 A healthy birth after intracytoplasmic sperm injection using ejaculated spermatozoa from a patient with Kartagener’s syndrome

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Introduction: Kartagener’s syndrome (KS) is characterized by the classic triad of chronic sinusitis, bronchiectasis and situs inversus. This is caused by dynein arm defects in axonemal microtubules in ciliary and flagellar structures. Male patients are invariably infertile because of the immotility of spermatozoa. We report a healthy birth that was achieved by intracytoplasmic sperm injection (ICSI) using ejaculated spermatozoa from a patient with KS.

Case report: The wife of the patient was a 33-year-old woman with infertility of one-year duration. Her diagnostic infertility work-ups were normal. Her husband was 33 years old. He had normal secondary sex characteristics and both testicu lar volumes were normal. His semen FSH, LH, testosterone and prolactin levels were all within normal limits. He had dextrocardia and dysosmia as well as a history of chronic bronchitis and sinusitis, the classic triad disorders of KS, and was therefore diagnosed with KS. Several semen analyses showed sperm concentration of 77-87 x 10^6/ml and ejaculated volume of 3.5-4.0 ml. However, only 2-28 motile spermatozoa were observed in the whole field with a Makler Counting Chamber. After eosin staining, 30% of the sperm were viable with unremarkable morphology. Transmission electron microscopy (TEM) of the sperm tail showed the absence of both inner and outer dynein arms. After counseling for this condition and suitability for ICSI, the couple proceeded with ICSI using ejaculated spermatozoa.

In August 2005, after ovarian stimulation by a long protocol using GnRHa / hMG, twenty-one oocytes were obtained, and 18 metaphase II oocytes were retrieved. Fresh semen samples were obtained after masturbation. The sperm concentration was 57.2 x 10^6/ml and the volume was 3.5 ml. Total motility was 0.3%, and no spermatozoa showed straight progressive motility. After swim-up preparation, we observed a few motile spermatozoa that appeared morphologically normal, and these motile spermatozoa were selected for ICSI. Thirteen oocytes were fertilized. All embryos were cryopreserved in the cleavage stage to prevent development of ovarian hyperstimulation syndrome.

In October 2005, two-step embryo transfer was performed in the hormone replacement (HR) cycle. A twin pregnancy was achieved, one resulting in missed abortion but the other being uneventful. A healthy female infant was delivered in June 2006 at a gestation age of 37 weeks and weighing 2675 g.

In April 2008, the couple was referred to our clinic for a second pregnancy. Four cycles of single blastocyst transfer using a cryopreserved-thawed blastocyst were repeatedly performed. Pregnancy tests were positive in three cycles, but all of the three pregnancies resulted in chemical abortion.

In January 2009, after using an ovarian stimulation protocol similar to that used in the first cycle, five metaphase II oocytes were retrieved. Sperm concentration and total motility were 68.1 x 10^6/ml and 0.1%, respectively, and a few progressive motile sperm were observed. The intracytoplasmic morphologically selected sperm injection (IMSI) procedure was performed with the progressive motile sperm. All oocytes were fertilized. One embryo was cryopreserved in the cleavage stage and the other four embryos were cultured for three more days, but no blastocyst was obtained. In March 2009, cryopreserved-thawed embryo transfer was performed in the HR cycle, but pregnancy was not achieved.

Conclusion: This case report demonstrates that successful pregnancy after ICSI in couples with Kartagener’s syndrome is possible with ejaculated spermatozoa. Our results suggest that even for KS patients, if motile sperm are observed, a fertilization rate comparable to that with ICSI of the other male factor can be expected. KS is a heterogeneous group of disorders with similar clinical presentations, and treatment should be individualized depending on sperm motility.

P-002 Sperm DNA decondensation is positively correlated with sperm morphological anomalies and negatively correlated with sperm motility

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Introduction: Sperm DNA is packaged very tightly to protect the DNA during the transit that occurs before fertilization. DNA damages are known to include impaired fertilization, poor embryonic development, high rate of miscarriage and morbidity on the offspring.

The aim of our retrospective study was to analyse the correlations existing between sperm DNA decondensation (SDI or sperm decondensation index) and several WHO sperm parameters, such as sperm count, motility, morphological anomalies and DNA fragmentation, according to the age of the infertile patients.

Material and Methods: Between June 2007 and April 2009, 1690 semen samples were collected, patients having signed an informed consent and approval from the ethic committee having been obtained. The sample were studied according to the WHO parameters (sperm concentration, motility and morphological anomalies rates), to DNA fragmentation levels and results were correlated with the SDI levels, taking into account patients’ age and abstinence duration. Sperm DNA fragmentation was evaluated by TUNEL assay (a result less than 30% was considered normal) and SDI by aniline blue assay (less than 20% was normal). Statistical analysis was performed using variance analysis and non parametric tests.

Results: Our results show a significant negative correlation between SDI and total motile sperm count (TMSC) where SDI of less than 20%, 20 to 29.9% and more than 30% were correlated to a TMSC of 28.1, 24.6 and 22 respectively (p < 0.05). Sperm morphological anomalies were closely and positively correlated with SDI (morphological anomalies rates of 82.1, 84.1, 87.5 respectively for the SDI levels mentioned above). However, there was no significant modification of SDI according to age, abstinence duration and DNA fragmentation.

Conclusions: Our study suggests that SDI is positively and closely correlated with sperm morphological anomalies. Moreover when total motile sperm count is low, SDI is significantly higher than 20%. However, sperm DNA decondensation varies independently of DNA fragmentation and of the patient age.

Sperm DNA decondensation should be evaluated in all infertile men with a high rate of sperm morphological anomalies and motility defects.

P-003 Is the outcome of chlamydial serology in seminal plasma and/or serum indicative of semen quality and sperm function during infertility investigation?

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Introduction: Chlamydia trachomatis is considered the most common sexually transmitted bacterial pathogen in industrial countries. The impact on female reproductive health is widely accepted, but the role of these microorganisms for male fertility is a matter of constant debate. In previous studies, no relationship of chlamydial (Chlam) serology results with semen quality was found, when a standard immunofluorescence-assay (IFA) was used to determine anti-chlamydial immunoglobulin (Ig) G-class antibodies (Ab) in serum. However, serologic assays are available, which allow to determine not only Chlam
The study also examined two different freezing protocols of human oocytes in these sperm studies. Sperm recovery and motility parameters were assessed. There was no medical indication of semen quality and sperm functional capacity. Patients' medical history and clinical andrological examination were taken into consideration as well as results of postcoital testing (PCT), and subsequent fertility (after control for female infertility factors).

Results: In total, 100 motile spermatozoa were frozen for research purposes. A total of 97 sperm were recovered, 49 (49/50; 98%) frozen in sucrose and 48 (48/50; 96.0%) in SpermFreeze, respectively. The survival rates were significantly higher when sperm were cryopreserved in sucrose (32/49; 65.3%) rather than SpermFreeze (17/48; 35.4%; p < 0.01). Sperm were recovered quickly (means 168 seconds in sucrose and 190 seconds in SpermFreeze, respectively), and easily.

Conclusion: CryoTop is a highly useful container for cryopreservation of single spermatozoa, and sucrose is an effective cryoprotectant. Our method can be considered as a quick, easy, and simple to cryopreservation of single spermatozoa.

P-005 Reproductive outcomes using Kruger's strict criteria in IUI cycles
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Introduction: It is well known that Intrauterine Insemination (IUI) cycles combined with ovarian stimulation along with induction of ovulation has become the first line of treatment for infertility. The aim of this study was to determine the effect of morphology as a seminal parameter in order to evaluate reproductive success in patients undergoing IUI in our facilities.

Materials and Methods: Retrospective study including 438 couples with unexplained infertility, undergoing IUI cycles. The period of the study ranges from December 2005 to September 2009. All patients were stimulated with rFSH (Puregon; Organon) starting cycle day 3, once ovarian quiescence was confirmed by transvaginal ultrasound scan, and estradiol and progesterone blood tests when needed. Starting dose ranged between 75 and 150 IU, depending on patients’ age and BMI. Final maturation was triggered with 250 mg of rhCG when at least one follicle reached 17 mm in mean diameter. Two IUI were scheduled at 16 and 28 hours since rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, and estradiol and progesterone blood tests were conducted at 16 and 28 hours since rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, and estradiol and progesterone blood tests were conducted at 16 and 28 hours since rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, and estradiol and progesterone blood tests were conducted at 16 and 28 hours since rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, and estradiol and progesterone blood tests were conducted at 16 and 28 hours since rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, and estradiol and progesterone blood tests were conducted at 16 and 28 hours since rhCG injection. Sperm samples were collected into a sterile container 2 h prior insemination, and estradiol and progesterone blood tests were conducted at 16 and 28 hours since rhCG injection.

Results: Although there is a trend towards better outcomes with increasing number of normal sperm, there were no statistically significant differences between both groups in terms of PR [A: 21.03% (82/390); B: 29.17% (14/48)] and MR [A: 2.31% (9/390); B: 2.08% (1/48)].

Conclusions: Sperm morphology is a widely used parameter to consider IUI. Our results indicated that, at least in our facilities, it does not predict IUI outcomes in terms of PR and MR. The narrow range of sperm morphology classification may be responsible of these results, although WHO criteria to classify routine sperm samples was classified into Group A (1-6% normal sperm) and Group B (7-14%). Pregnancy Rate (PR) and Miscarriage Rate (MR) were compared in both groups. t-test was applied.

P-006 Oral anti-oxidant use for male partners of couples undergoing fertility treatments
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Introduction: Between 30%-80% of male subfertility cases are considered to be due to the damaging effects of oxidative stress on sperm. Oral supplementation with antioxidants may improve sperm quality by reducing oxidative stress. This Cochrane review aimed to evaluate the effect of oral supplementation with antioxidants on male partners of couples attending a fertility clinic.
Materials and Methods: All RCTs of oral antioxidant supplements in men were searched in the following sources: the Cochrane Menstrual Disorders and Subfertility Group Register, MEDLINE, CENTRAL, EMBASE, CINAHL, PSYCINFO and AMED databases (from their inception until January 2010), trial registers, unpublished literature, reference lists and experts in the field. RCTs comparing any type or dose of antioxidant (single or combined) versus placebo, no treatment or another antioxidant that were taken by the male partner of a couple seeking fertility assistance were included. The outcomes were live birth, pregnancy, miscarriage, or spontaneous abortion, stillbirth, level of sperm DNA damage, sperm motility, sperm concentration and adverse effects.

We performed statistical meta-analyses in accordance with the guidelines developed by The Cochrane Collaboration for the effect of antioxidant/s versus placebo per couple randomised.

Results: Fifty trials were considered and 32 met the inclusion criteria. 2696 couples in total.

Live birth: Two trials reported live birth. The use of antioxidants in men compared to placebo was associated with an increased live birth rate (pooled odds ratio (OR) 6.44, (1.72 to 24.04, F = 0%, p < 0.006)). This result was based on 10 live births from a total of 117 couples in the two studies. One of these trials included couples undergoing IVF.

Pregnancy rate: There were 79 pregnancies in 11 trials including 795 couples. Antioxidant use compared to placebo was associated with an increased pregnancy rate (pooled OR 3.89 (2.33 to 6.39, F = 0%, p < 0.00001)). Sensitivity analysis on two trials that included couples undergoing IVF showed that the use of antioxidant remains associated with increased pregnancy rate (pooled OR 4.22, (2.33 to 7.63, F = 0%, p < 0.00001)).

Miscarriage rate: There was no evidence of an effect on miscarriage rates, (pooled OR 1.15 (0.21 to 6.28; p = 0.87)) between the antioxidant and placebo groups in two trials, 145 couples.

Still Birth: There were no trials reporting stillbirth.

DNA fragmentation: One trial reported DNA fragmentation. There was a difference (OR -13.80, (-17.50 to -10.10; P = 0.000). Antioxidant use compared to placebo was associated with a reduction in DNA fragmentation.

Sperm concentration antioxidant use compared to placebo was associated with an improvement in sperm concentration within the following timeframes:
1. at ≤3 months: pooled OR 9.88 (7.17 to 12.59; F = 52%, p < 0.00001). 348 participants studied in seven trials.
2. at 6 months pooled OR 4.19 (3.81 to 4.56; F = 89%, p < 0.00001). 915 participants studied in seven trials.
3. at ≥9 months: pooled OR 1.38, (0.81 to 1.95; F = 64%, p < 0.00001). 332 participants in three trials.

Side effects: No studies reported evidence of harmful side effects of the antioxidant therapy used.

Conclusions: There is some evidence that antioxidant supplementation in subfertile males may improve the outcomes of live birth, pregnancy rate and sperm parameters for subfertile couples.

P-008 Variations in folate pathway genes are associated with male infertility
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Introduction: Folate gene polymorphisms have been previously related with reproduction disorders. Recently several association studies have suggested that polymorphic variants in the MTHFR gene may be associated with reduced sperm counts in the human leading to male infertility in some populations. In the present study we have analyzed 19 polymorphisms from 13 genes of folate cycle in fertile males.

Materials and Methods: A group of 28 fertile men (classified according to WHO and Kruger criteria) and 122 controls were genotyped for the following
Participants (n = 375) were partners of pregnant women and their partners (FAI) as well as the ratio FAI/LH. Semen analyses were performed according to WHO criteria. Pearson correlations and parametric tests were used for unadjusted analyses. Multiple linear regression analyses were used to examine associations controlling for age, body mass index, smoking, ethnicity, urinary creatinine concentration, time of sample collection and duration of abstinence.

Results: After multivariate adjustment, we observed no associations between any semen parameter and urinary BPA concentration. However, a significant inverse association was found between urinary BPA concentration and FAI levels and the FAI/LH ratio, as well as a significant positive association between BPA and SHBG.

Conclusions: Our results suggest that, in fertile men, exposure to low environmental levels of BPA may be associated with a modest reduction in markers of free testosterone, but any effects on reproductive function are likely to be small, and of uncertain clinical significance.

P-010 Morphologically selected sperm injection as a routine and in vitro fertilization outcomes

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Introduction: The value of spermatozoa head morphology and its relation to in vitro fertilization outcomes has been reported in several studies. Morphological selection of spermatozoa under a high magnification (IMSI) has been introduced to provide a better clinical outcome than the conventional selection method (ICSI). However, most studies report the effect of IMSI only on patients with previous repeated ICSI failures. This retrospective study was conducted to compare results of two methods used for selection of spermatozoa, to be injected, in intracytoplasmatic sperm injection procedures and to evaluate possible advantages of using the IMSI technique, as a routine, for all patients, in our center.

Material and Methods: This study was performed in a private assisted reproduction center in Brazil (Genesis – Centro de Reprodução Humana – Passo Fundo, RS). Only couples with female age ≤ 39 years, more than 6 MII (mature) oocytes collected and with ejaculated sperm were selected. 118 cycles of intracytoplasmatic sperm injection have been analyzed. The cycles were divided into two groups (A and B). In group A (59 cycles), employed, to assess the morphology of sperm to be injected, the conventional equipment that provides optic magnification of 400X (conventional ICSI). In group B (59 cycles), were employed an equipment that provides an optic magnification of 6800X (IMSI). In each group, couples were categorized into 2 subgroups, according to the presence of male factor (subgroup MF), or not (subgroup NMF). The female age among all groups and subgroups were similar. The same embryologist performed all laboratory procedures (ICSI and IMSI), as well as the same physician performed all embryo transfers.

Results: The fertilization rate was similar in group A (ICSI: 77.07%) and B (IMSI: 79.10%), as well as the implantation rate (ICSI: 22.29%, IMSI: 28.39%), however, the clinical pregnancy rate was significantly higher (P < 0.05) in group B (ICSI: 52.54%, 31/59) than in group A (ICSI: 35.59%, 21/59). Evaluating the fertilization rate (ICSI: 79.09%, IMSI: 77.18%) and implantation rate (ICSI: 20.83%, IMSI: 29.17%), the clinical pregnancy rate was significantly higher (P < 0.05) in group B (IMSI: 60.71%, 17/28) than in group A (ICSI: 32.14%, 9/28).

Conclusions: This report of outcome comparison between the IMSI and the ICSI demonstrates that the use of spermatozoa morphologically selected for injection (IMSI), as a routine, provides significant positive impact in clinical pregnancy rates, mainly in the presence of male factor. In the absence of male factor, there were no data enough to support the real advantage of this technique comparing with conventional ICSI.

P-009 Are environmental levels of bisphenol A associated with reproductive function in fertile men?

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Introduction: Widely used man-made chemicals, including bisphenol A (BPA), can induce hormonal alterations through a variety of cellular and molecular mechanisms. Several rodent and in vitro studies have consistently demonstrated the estrogenic effect of BPA. However, few studies have examined the relationship between human exposure to BPA and levels of male reproductive hormones, and none has examined semen parameters and BPA. Our objective was to investigate the relationships between environmental BPA exposure and reproductive parameters in prospectively recruited, well-characterized, fertile men.

Material and Methods: Participants (n = 375) were partners of pregnant women who participated in the Study for Future Families in four US cities and provided blood, semen and urine samples on the same day. BPA was measured in urine. Serum samples were analyzed for reproductive hormones, including follicle-stimulating hormone, luteinizing hormone (LH), testosterone, inhibin B, estradiol and sex hormone-binding globulin (SHBG), the free androgen index (FAI) as well as the ratio FAI/LH. Semen analyses were performed according to WHO criteria. Pearson correlations and parametric tests were used for unadjusted analyses. Multiple linear regression analyses were used to examine associations controlling for age, body mass index, smoking, ethnicity, urinary creatinine concentration, time of sample collection and duration of abstinence.

Results: After multivariate adjustment, we observed no associations between any semen parameter and urinary BPA concentration. However, a significant inverse association was found between urinary BPA concentration and FAI levels and the FAI/LH ratio, as well as a significant positive association between BPA and SHBG.

Conclusions: Our results suggest that, in fertile men, exposure to low environmental levels of BPA may be associated with a modest reduction in markers of free testosterone, but any effects on reproductive function are likely to be small, and of uncertain clinical significance.

P-011 A simple method for cryopreservation of small numbers of human spermatozoa

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Introduction: Men with severe oligozoospermia can be treated using surgical isolation of spermatozoa from testicles, although recovery rate is variable. The...
need to repeat testicular operations can be avoided by the use of sperm cryopreservation. But conventional cryopreservation procedure is regularly associated with the loss of spermatozoa. Placing very low numbers of spermatozoa in specific containers may eliminate these risks. In these 12 years, various types of containers including empty zona pellucidae, droplets on plastic dish, mini-straws, micropipettes, cryoloops, copper loops, volvox globulator algae, agarose gel microspheres and algic acid drops have been reported by many authors. However there is a lack of ideal container that could be used universally. In this study we tried to cryopreserve small numbers of spermatozoa using new freeze-drying containers consisting of a tray and cryotube (Cell Sleeper).

Materials and Methods: Spermatozoa (n = 50) from 10 fertile men with normal sperm count were used to evaluate the efficiency of the method. Ejaculates were processed through density gradients. Individual motile spermatozoa were selected from discarded supernatant and put in the microdroplets with 7% PVP solution using the ICSI pipettes. For single sperm cryopreservation, 5 spermatozoa were then deposited in 3.5-µl microdroplets of sperm freezing medium on the trays. The sperm freezing medium consisted of a 10:7 solution, HEPES-buffered HFF99 medium (Fuso Pharmaceutical Ind., Japan) + 30% synthetic serum substitute (Irvine Science, USA) and SpermFreeze (FertiPro, Bergium). To prevent the generation of convection in microdroplets, all micromanipulations were performed at room temperature (stage heaters were switched off). These trays were put into cryotubes without being overlaid with oil. They were placed horizontally 0.5 cm above liquid nitrogen for 2.5 minutes, followed by plunging in it. Cell Sleepers were mounted to the same types of canes with regular cryotube and then stored. On thawing, the frozen tubes were transferred to room temperature for 1 minute and then trays were taken out of the cryotubes. The trays were overlaid with oil and transferred to 37°C hot plate for 1 minute. Sperm samples were observed by inverted microscope at X100-400 magnification at room temperature (stage heaters were switched off). Motile spermatozoa were transferred to droplets of medium using ICSI pipettes. Immotile spermatozoa were transferred to droplets of water to evaluate the viability by hypo-osmotic swelling test. The post-thaw sperm recovery, motility and viability rates were analyzed. There was no medical fertilization of human oocytes in this research.

Results: Total of 50 spermatozoa was frozen for research purposes. A total of 50 frozen spermatozoa were recovered from 10 containers successfully, resulting in an overall recovery rate of 100%. The post-thaw motility and viability rates were 54% and 70%, respectively. The average time required for recovering all 5 spermatozoa per tray was 11 minutes and 17 seconds.

Conclusion: The results of this preliminary study suggest that our method using Cell Sleeper was an easy and effective method for recovering spermatozoa after thawing in cryopreservation of very small numbers of human spermatozoa.

P-012 Determination of the surface pathology of human sperm by atomic force microscopy
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Introduction: Atomic force microscopy (AFM) employs a sharp probe for profiling surfaces with unique resolution. This technique has developed into an invaluable multidisciplinary tool for advanced characterization of different samples. In its basic application, AFM provides high-resolution imaging of surface structures at scales ranging from a few nanometers to hundreds micrometers. Oxidative stress is considered one of the basic etiological factors of the male’s fertility disorders. Excessive production of oxygen radicals results in damage to the sperm’s cell membrane and to the DNA. The aim of the study was to use AFM for imaging the surface pathology of the sperm exposed to experimental oxidative stress.

Material and Methods: Fresh samples of semen from sperm donors were obtained. Spermatozoa were selected by using the conventional swim-up procedure and dried samples on glass plates prepared. The slides were transported to nanobiotechnology laboratory and scanned by AFM. Atomic Force Microscope NTegra Vita (NT-MDT, Moscow, Russia) was used to characterize spermatozoa morphology within a nanometer scale. Scanning head type SFC050LNTF designed for scanning by probe (maximal operation area 100x100x10 μm)

equipped with a holder for operation in a liquid was used for all experiments. Glass slide with immobilized spermatozoa was fixed to the bottom of plastic Petri dish and the internal volume was filled with sterile isotonic solution of sodium chloride. Samples imaged in contact mode at ambient temperature were scanned by using silicon nitride probe (Vecco Probes) with 0.01 N/m elastic constant at 0.25 Hz scanning speed. Surface topography and spatial distribution of electrical potential gradient on the sample surface were measured and recorded simultaneously, thus providing more complex understanding to the surface morphology. After first scanning and imaging of the normal spermatozoa, the slides were exposed to the hydrogen peroxide solution (3%) for 10 minutes, properly rinsed with distilled water, air dried and re-scanned. The defects were detected and recorded.

Results: The obtained images clearly show both normal sperm head and defects of the surface of spermatozoa exposed to hydrogen peroxide. Even the ultrastructure at the top of the flagellum and the region of the acrosome cap are clearly distinguishable. Moreover, elements of internal structure can be observed whenever the plasmatic membrane is missing. The best images of both normal and damaged spermatozoa were selected for presentation.

Conclusion: The collected AFM images clearly highlight many details of normal spermatozoa and spermatozoa damaged by hydrogen peroxide. This technique could be an important tool in the research of oxidative stress and understanding its effect on male infertility.

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P-013 Influence of male obesity on pregnancy rates in couples undergoing oocyte donation
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Introduction: Evidence has been reported on the relation between obesity and reduced fertility in males. Research has focused on assessing the influence of male obesity on the quality of sperm, proving a correlation between an increase in body weight and alteration in sperm parameters. The influence of male obesity on time to pregnancy has also been demonstrated, indirectly relating the effect of male obesity on couple infertility.

Thus, in view of the absence of works showing the direct relationship between male obesity and sterility, our aim is to assess the effect of male obesity on pregnancy rates in couples undergoing undergoing oocyte donation. This study was performed in a fertility clinic and included 1931 couples. The reason for oocyte donation was the lack of gametes to create a pregnancy. We selected this group minimizes the possible role of the female factor in conjugal sterility, thus allowing for a better assessment of the role of semen in pregnancy rates.

Material and Methods: We examined the association between male partner obesity and fertility among 1931 couples who underwent an oocyte donation cycle in our clinic within 2007 and 2008. Obesity has been calculated using the body mass index (BMI). We categorized BMI (kg/m²) as normal weight (20–24), overweight (25–29) and obese (³30). The pregnancy rate (PR) and implantation rate (IR) were divided into three groups based on the male BMI. Sperm parameters were also analysed to assess the relationship between male BMI and semen quality. Recipients with detrimental reproductive factors such as age over 45 years, heavy smokers, BMI >30 and uterine pathology were excluded.

Results: PR and IR in the normal weight group were 49.4% and 31.4%, 52.8% and 31.5% in the overweight group and 51.1% and 33.8% in the obese group. After adjustment for all covariates, PR RR associated with overweight and obese men were 1.06 (95% CI = 0.96–1.18) and 1.03 (95% CI = 0.87–1.21), respectively, compared with normal weight men. The IR RR in the overweight and obese men were 1.00 (95% CI = 0.85–1.17) and 1.07 (95% CI = 0.84–1.36), compared with the normal weight group.

These results indicate no difference in the pregnancy and implantation rates between the three groups.

However when comparing sperm characteristics obese men did have lower sperm concentrations than those of normal and overweight men (IMC >30: 44.8 mill/ml; IMC 25-30: 52.3 mill/ml; IMC <25: 52.8 mill/ml; ³ vs 2; p <0.05).

There was no relation between increasing male BMI and percent of motile sperm or abnormal sperm morphology.
Conclusions: Our study found an association within obesity and poor semen quality but not with worse outcomes in ART with donated oocytes. These results may suggest that male obesity is not an important independent factor determining infertility.

P-014 Dynamics of sperm DNA fragmentation in fresh and processed samples used for ICSI cycles
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Introduction: Sperm DNA fragmentation has been associated with a decrease in natural conception and with failures in cycle outcome in couples undergoing assisted reproduction techniques (ART). It can be caused by intrinsic (i.e., testicular apoptosis) or extrinsic factors, such as the iatrogenic damage during the manipulation of sperm samples along ART procedures. Indeed, in most of the cases, fresh or capacitated semen samples are maintained in the incubators for different periods of time until they are used for ART. As sperm DNA degradation is a non-static process, the aim of this study was to evaluate the dynamic of sperm DNA fragmentation in semen samples from infertile men before and after its preparation for the use in ICSI cycles.

Materials and Methods: Human semen samples from 37 patients were obtained by masturbation after 2-3 days of sexual abstinence. After liquefaction, semen analysis was performed following WHO criteria, and then samples were divided in two identical portions. One of them was maintained at 37°C and at different time points (0h, 1h, 3h, 5h, 24h) an aliquot was taken to perform sperm DNA fragmentation assays. The other part of the semen samples was used to isolate progressive motile sperm cells by using discontinuous Sperm Grad gradients. After density gradient separation, a second evaluation of sperm parameters was assessed and then processed spermatozoa were maintained at 37°C until different aliquots were used for ICSI cycles and sperm DNA fragmentation assays at different time points (0h, 3h, 24h after sperm cell isolation). Assessment of sperm DNA fragmentation was performed by using the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridinetriphosphate nick-end labelling (TUNEL) assay using a commercial kit (Roche) and following manufacturer’s recommendations. The samples were then analyzed by flow cytometry using FACSscan cytometer. Statistical analyses were performed using SPSS software. A p value < 0.05 was considered statistically significant.

Results: At t = 0h, capacitated semen samples showed a significant increased DNA fragmentation levels compared to fresh spermatozoa (11.5 % ± 9.5 vs 22.9% ± 19, respectively, p = 0.002). The kinetic study also demonstrated an increase in DNA fragmentation levels both in fresh (t 1h = 14.7% ± 11.4; t 3h = 19.1% ± 12.3; t 5h = 21.0% ± 11.3; t 24h = 23.8% ± 12.7) and in capacitated (t 3h = 26.2% ± 23.5; t 24h = 27.6% ± 25.4) sperm cells. Interestingly, the rate of increase of the frequency of sperm DNA fragmentation was significantly lower in capacitated samples compared to fresh ones (1.14 vs 1.66 of increment rate at 3h, respectively), probably due to the lower rate of sperm dead cells in the samples.

Conclusions: Obtained results showed a significant incidence over time of sperm DNA fragmentation, suggesting that the use of semen samples for ART procedures must be as short as possible to reduce sperm DNA fragmentation.

P-015 The effects of severity of oligozoospermia on intracytoplasmic sperm injection (ICSI) cycle outcome
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Introduction: The development of intracytoplasmic sperm injection (ICSI) created a new era in the field of assisted reproduction and revolutionized the assisted reproductive treatment (ART) protocols for couples with male factor infertility. In general, in the case of male subfertility, ICSI treatment results in higher fertilization rates per oocyte compared with conventional IVF treatment. Even the most severe cases of oligo-asthenro-teratozoospermia (OAT) can now be successfully treated with ICSI. A dominant effect of a single suboptimal semen parameter on the fertilization results after IVF and ICSI was reported for sperm morphology and motility. However, only a few studies have mentioned semen quality (concentration and motility) in connection with the efficacy of ICSI performed with ejaculated spermatozoa. We determined whether the sperm concentration and motility in cases of oligozoospermia are associated with laboratory or clinical outcomes. The aim of this study was to explore the relationship between sperm quality and embryo development, pregnancy, and implantation rates as well as fetal loss in patients undergoing ICSI.

Materials and Methods: In this case study, we retrospectively analyzed data obtained from a total of 908 ICSI-ET cycles performed with fresh ejaculated spermatozoa. To exclude poor-responding patients, who would influence the results, couples in which the wife was older than 38 years were excluded. Then, the patients were divided into four treatment groups according to the results of an analysis of the husband’s semen: (A) mild oligozoospermia (10 ≤ Co. < 20 × 10^6/ml, n = 283), (B) mild to severe oligozoospermia (5 ≤ Co. < 10 × 10^6/ml, n = 192), (C) severe oligozoospermia (1 ≤ Co. < 5 × 10^6/ml, n = 259), and (D) very severe oligozoospermia (0 < Co. < 1 × 10^6/ml, n = 174).

After 2-3 days of sexual abstinence, semen samples were produced by masturbation. All ejaculate-related ICSI procedures used fresh (not frozen) ejaculated spermatozoa. During oocyte retrieval, the patients were stimulated using standard GnRH agonist / FSH protocols. Fertilization was considered normal when two clearly distinct pronuclei (PN) containing nucleioli were present. The embryo cleavage of the 2PN oocytes was evaluated after a further 24 h of in-vitro culture (day 2). The embryos were scored according to the quality, number, and size of the blastomeres and the percentage of anucleate fragments. Cleavage-stage embryos were graded according to the criteria set out by Veeck. Clinical pregnancy was confirmed when the development of a gestational sac was observed by means of ecographic screening at 7 weeks of pregnancy and the presence of a fetal heartbeat, and the implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred.

Results: There were no significant differences in the background of the wife’s age, the evaluation of normal cleaved embryos (% of 2PN oocytes), and high quality embryos on day2 (% of cleaved embryos) after ICSI among the groups. However, the very severe oligozoospermia group (D) demonstrated a significantly lower percentage of 2PN oocytes compared with the other groups (73.6%, 78.4%, 71.4%, and 63.9%, Group A, B, and C vs. Group D, p < 0.05). There was no significant difference in clinical pregnancy rates (38.2%, 38.0%, 36.7%, and 32.8%, respectively), implantation rates (24.5%, 21.4%, 21.9%, and 21.4%, respectively), or fetal loss (17.6%, 20.5%, 19.3%, and 19.3%, respectively). Even when the treatment groups were selected based on sperm motility of ≤ 40%, there were significant differences in zygote production (71.1%, 71.3%, 70.7%, and 63.8%, respectively, p < 0.05) but not clinical pregnancy rates (36.6%, 31.5%, 37.5%, and 33.3%, respectively) among Group A, B, and C vs. Group D. In addition, no significant differences in the production of clinical pregnancy rates were detected between motility of < 40% and ≥ 40% in any group.

Conclusions: Our data indicates the importance of selecting good quality sperm for oocyte injection especially in very severe oligozoospermia.

P-016 Follicle-stimulating hormone induces meiosis of immortalized germ cells and decreases secretion of TGF-beta2
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Introduction: Spermatogenesis and Sertoli cell (SC) functions are dependent upon the actions of testosterone and follicle-stimulating hormone (FSH). Proliferation of spermatogonia and entry into meiosis are induced by FSH, whereas testosterone is mainly involved in post-meiotic events. The hormonal effects on germ cell (GC) differentiation are mediated indirectly by Sertoli cells via cell-cell contacts or in a paracrine manner. FSH exerts its effects via high-affinity membrane receptors (FSH-R) but also increases calcium influx and cAMP signaling, thus leading to stimulation of gene expression and protein synthesis of Sertoli cells. Up to date, only few genes have been described to be regulated by FSH, but intriguingly mRNA expression of transforming growth factor (TGF)-betas in the testis ceases under the influence of hormones. However,
investigation of endocrine effects on immortalized Sertoli cells is hampered by inactivation of hormone receptors in cell culture. Thus, the present study aimed to establish an in vitro system of FSH-sensitive Sertoli cells cocultured with germ cells and to investigate the hormonal effects on both cell types.

**Materials and Methods:** FSH-receptor positive and FSH-R-negative Sertoli cell lines were cocultured with immortalized germ cells (consisting mainly of spermatocytes) and were stimulated with recombinant FSH of up to several weeks. Differentiation of GCs into spermatids was analyzed with stage-specific markers (e.g. transition proteins) and flow cytometry. Treatments with forskolin were used to mimic FSH effects in FSH-negative SCs. Secretion of TGF-beta2 was quantified with an ELISA.

**Results:** Coculturing of SCs and GCs results in cord formation which was severely attenuated by FSH. Differentiation of immortalized germ cells into round spermatids occurs after approximately two weeks. Although germ cell differentiation also happened spontaneously FSH as well as forskolin induced differentiation more strongly. Only in hormone-sensitive Sertoli cells FSH reduced secretion of TGF-beta2 significantly. In contrast forskolin lowered TGFbeta2 secretion also in hormone-insensitive Sertoli cells. Of note, the Sertoli cells secreted only the inactive form of TGF-beta2.

**Conclusions:** We have successfully established an in vitro system for studying induction of meiosis of germ cells in vitro. Furthermore FSH effects on Sertoli cells and thus on germ cell differentiation can now be studied in more details.

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**P-017 Sperm DNA fragmentation measured by the alkaline Comet assay is an independent factor to predict IVF success for couples with male infertility**

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**Introduction:** Infertility affects one in six couples in Europe with dysfunction- al sperm being one of the most common causes. Conventional semen analysis lacks power as a prognostic test for assisted reproductive treatment (ART). In contrast, sperm DNA damage is closely associated with fertilization, embryo quality and implantation so it has the potential to be a useful prognostic test. Although sperm DNA fragmentation tests show much promise, they have not yet become routine in the clinical setting. The primary objective of this study is to present for the first time the predictive power of sperm DNA damage in couples presenting with male infertility using the sensitive Comet assay.

**Materials and Methods:** In this study, men attending for IVF (n = 70) and fertile donors (n = 28) were recruited. Semen analysis was evaluated according to WHO guidelines (1992) and sperm prepared by density gradient centrifugation (DGC) was analysed using the alkaline Comet assay. The fertilization rate (FR) was calculated based on the percentage of oocytes fertilized while the embryo cumulative score (ECS) was calculated by multiplying embryo quality (A = 4, B-3, C = 2) with the number of blastomeres/embryo and when a patient had more than one embryo, a mean across embryos were calculated to obtain total quality of all embryos generated. This was calculated for the total number of embryos/treatment (ECS) and for embryos transferred (ECS.). Clinical pregnancy was determined by the identification of a gestational sac after 7 weeks. Based on predictive probabilities, a threshold value was obtained with 95% power to diagnosis male infertility and to determine clinical pregnancy after ART.

**Results:** DNA fragmentation in sperm from fertile men was 12.5 ± 1.7% whereas that from infertile men was 57.9 ± 2.7%. We calculated the odds ratios using DNA damage of sperm from fertile donors and IVF patients as 117.3 (12.73-2731.83) with an area under the ROC curve of 0.97 cm² and 0.88 cm², respectively.

**Conclusions:** The alkaline Comet assay is a useful method for diagnosis of male infertility and prediction of fertilization, embryo quality and pregnancy rates in IVF.

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**P-018 Effect of HIV-1/HCV infection and sperm washing treatment in sperm DNA fragmentation**

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**Introduction:** Both human immunodeficiency virus (HIV-1) and Hepatitis C virus (HCV) cause sexual transmission diseases (STD) with vertical transmission risk. Sperm- washing techniques have allowed serodiscordant men undergoing assisted reproductive techniques (ART) preventing horizontal and vertical transmission to their partners and children. It has been demonstrated that both viral infections and iatrogenic damage (like sperm manipulation techniques) increase sperm DNA fragmentation. The aim of this study was to analyze the sperm DNA fragmentation in HIV-1 and HCV infected patients that washed their sperm samples and to evaluate if this damage is due to the viral infection or to the sperm manipulation during the washing technique.

**Materials and Methods:** Human semen samples from 12 patients infected with HIV and/or HCV and 10 healthy donors were obtained by masturbation after a sexual abstinence of 3 days. After liquefaction, semen analysis was performed following WHO criteria. The sperm was washed following the conventional techniques (different washes with HTF medium and 1-2 swim-up/density gradient capacitations) and cryopreserved using freezing medium (TYP) until the detection of HCV/HIV-1 RNA and proviral DNA by nested polymerase chain reaction (PCR) assay. To analyze the sperm DNA fragmentation 3 aliquots were taken from fresh samples (to study the damage that HIV/HCV may cause to the DNA), washed samples (to observe the iatrogenic damage) and finally a thawed aliquot (to analyze the iatrogenic and the damage after cryopreservation). The method used was the terminal deoxynucleotidyl transferase-mediated UTP nick-end labelling (TUNEL assay) using a commercial kit (Roche). Briefly, fixed sperm samples (PFA 4% PBS) were washed twice with HTF and incubated with Tdt enzyme (terminal deoxynucleotidyl transferase). Percentage of DNA-fragmented cells was analyzed by flow cytometry.

**Results:** The samples used as controls have similar seminal parameters that serodiscordant patients samples. Preliminary data showed a higher percentage of sperm DNA fragmentation in fresh samples from those patients presenting a viral infection than in healthy donors used as controls. After washing sperm technique sperm DNA fragmentation significantly increased indicating that the technique could be aggressive and can have a harmful effect on the sperm DNA. Cryopreservation procedure has also a negative effect in the sperm DNA, since DNA fragmentation is higher in thawed samples than before cryopreservation.

Further studies are necessary in order to evaluate if the washed sperm technique has any incidence in the pregnancy rate.

**Conclusions:** Seropositive men present a higher percentage sperm DNA fragmentation than healthy men due to the viral infection and this damage is deteriorated after wash and cryopreserve the sample. Although it has been demonstrated that the sperm wash technique is aggressive further studies must be done in order to know the impact on pregnancy rate.

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**P-019 Effects of sperm progressive motility and DNA damage on fertilization rates in vitro**

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We gratefully acknowledge Hamilton Thorne Biosciences, Beverly, MA 01915-6143, USA for funding the project.
Introduction: One in six couples experiences infertility problems so assisted reproductive technologies have a major role in today’s society. In vitro fertilization (IVF) outcomes are generally poor in patients with male infertility, with low fertilization rates and hence fewer embryos are available for transfer. Sperm motility is important for the sperm to fertilize the oocyte in vitro, so this has been considered an important factor in IVF. Similarly, sperm DNA damage is also shown to have a negative association on fertilization rates. The aim of this study is to determine the effect of sperm progressive motility and sperm DNA damage on in vitro fertilization rates.

Materials and Methods: A total of 216 couples attending for IVF were recruited and of these 136 couples were included in this study with the following criteria a) a minimum of five oocytes available for treatment, b) female partners < 40 years. In addition, couples with failed fertilization and men with anti-sperm antibodies in their semen were excluded from the study. Semen analysis was performed according to WHO guidelines (1992). Native semen and sperm prepared by density gradient centrifugation were analysed for DNA damage by the alkaline Comet assay. The fertilization rate (FR) was calculated as the percentage of oocytes fertilized. Pearson correlation was used to find any association between the variables; progressive motility, DNA damage and FR. Chi-square analysis was performed for each of the three variables separately. Each variable was categorised into low (Group A: 0-40%), moderate (Group B: 41-70%) and high (Group C: 71-100%).

Results: An overall FR of 66.1% was observed from 1,733 eggs included in the study. Among the conventional semen parameters there was a significant correlation only between progressive motility and FR ($r^2 = 0.189; P = 0.011$). A significant negative correlation was observed between sperm DNA damage and FR ($r^2 = 0.302; P < 0.001$). When divided into the three categories, high and moderate motility groups (C and B) had high FR 66% and 61% respectively, while low motility (A) had 48% FR. In the case of sperm DNA damage, low DNA damage group (A) had 76% FR, while groups B and C had 58% and 44% FR respectively. The odds ratio to determine fertilization using sperm motility was 4.81 (1.89-12.65) while the odds ratio was 24.18 (5.21-154.51) using sperm DNA fragmentation. Sperm of men with high DNA damage and low motility results in 9.5 times and 2.6 times the increased relative risk of lower fertilization (< 40%), when compared with low DNA damage and poor motility categories. Similarly, sperm DNA damage showed a higher specificity (93.3%) in predicting FR than progressive motility (77.8%). Sperm with high progressive motility and low sperm DNA damage had 96.0% probability of resulting in >70% FR.

Conclusion: Sperm progressive motility and sperm DNA damage are independent predictors of in vitro fertilization rate. However, measurement of sperm DNA damage by alkaline Comet assay has greater specificity than the conventional measurement of sperm abnormalities in predicting fertilization in vitro. This study shows the usefulness of sperm DNA testing in predicting fertilization rates in IVF.

We gratefully acknowledge Hamilton Thorne Biosciences, Beverly, MA 01915-6143, USA for funding the project.

P-021 Sperm DNA Fragmentation: the effect of age
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Introduction: During the last decades, many studies have shown the negative effect of age on female fertility. However, it is still controversial if male fertility is also affected. There are studies which associate spermatozoa with alteration in chromatin structure, with poor ART outcome, whilst others suggests that high sperm DNA fragmentation can compromise embryo viability resulting in pregnancy loss. There is significant difference between proven fertile and sub-fertile or infertility men, and the susceptibility to denaturation of their nuclear DNA. The aim of this study is to evaluate the effect, if any, of age on sperm DNA fragmentation.

Material and Method: 579 patients, between 23 and 71 years, who were tested for sperm DNA fragmentation using flow cytometry assay and Acridine orange test, between Jan 2007 and Dec 2009 were included in the study. Test results with DNA fragmentation index >15% (high fragmentation rate) were considered abnormal, and were analyzed with a logistic regression analysis in order to evaluate the effect of the age on sperm DNA fragmentation.

Results: 473 patients (81.7%) had a normal test, and 106 (18.3%) were abnormal. Abnormal test results, by age category are as follows: < 35 years 11.5% (31/268 patients); 35-40 years 19.3% (30/155 patients); 41-50 years 24.2% (33/132 patients); >50 years 50% (12/24 patients). When logistic regression analysis was applied, the odds ratio (ORs) for age and sperm DNA fragmentation was 1.08 per life year [95% Cis 1.06-1.10] (p value < 0.05) (Hosmer Lemeshow:0.7539).

Conclusion: Sperm DNA fragmentation increases with age, and should be considered when evaluating couples with infertility.

P-020 Magnetic activated cell sorting (MACS) improves oocyte donation results associated to severe male factor infertility
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Introduction: Previous results obtained by our group on fresh egg-donor intracytoplasmic sperm injection (ICSI) cycles have shown that overall mean embryo development, implantation, and pregnancy rates differed significantly among couples with severely altered semen parameters from those with normal or moderate semen abnormalities. A possible cause, albeit hypothetical, for the difference observed between both groups could be explained by the inclusion of apoptotic sperm during ICSI. Magnetic activated cell sorting (MACS) is a novel sperm preparation technique that separates apoptotic from non-apoptotic spermatozoa based on the expression of phosphatidylserin, and several studies have already shown an improvement on semen quality by eliminating apoptotic sperm. Oocyte donation offers a powerful tool for the study of sperm quality, since oocyte and endometrial characteristics can be controlled and are comparable among the recipients. The aim of this prospective study was to assess the impact of the MACS technique, selecting non-apoptotic spermatozoa, on the outcome of ICSI-egg donation cycles.

Material and Methods: Seventy two fresh egg donor cycles associated to severely altered sperm parameters and which fulfilled specific criteria were included in this study. These criteria were: a) cycles in which >3 embryos were yielded and at least two Day 3 good quality embryos were available for embryo transfer (ET), and b) sperm analysis which included one or more of the following findings: normal morphology by strict Kruger’s criteria (< 4%, sperm concentration <10million/ml, progressive motility <30%, and post-preparation total motile count < 5 million. Eighteen couples were treated by ICSI following sperm sample preparation by MACS (study group), while 54 couples (control group) underwent ICSI with sperm sample preparation by density gradient centrifugation (DGC) only. Fertilization rate, cleavage, embryo quality (evaluated on Day 3), and pregnancy and implantation rates were evaluated.

Results: Normal sperm morphology improved after MACS selection. The number of fertilized oocytes and the number of Day 3 embryos >7 cells was similar between both groups. However, the number of good quality embryos available for ET was significantly lower in the control group ($P < 0.005$). Pregnancy rate (PR) was higher and miscarriage rate (MR) was lower in the study group compared to the control group (PR 66.66%12/18 vs. 29.63% 16/54 and MR 16.66% 2/12 vs 18.75% 3/16, respectively). Furthermore, a slightly higher implantation rate was also observed in the study group (MACS) compared to the control group (DGC) (IP 26.92% 14/52 vs. 11.92% 18/151).

Conclusions: Our results suggest that integrating MACS to the sperm preparation technique, in those couples with male factor infertility, may improve semen quality and pregnancy rate by eliminating apoptotic sperm. We believe that our findings should be taken into consideration when evaluating and counseling patients who are willing to undergo donor oocyte assisted conception, since ICSI alone may not be able to select the highest quality sperm.

P-022 Impact of male or female gametes on embryo quality in couples with idiopathic infertility
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Introduction: Up to 30% of infertile couples present with idiopathic infertility. In these patients this diagnosis is made after standard infertility evaluation fails to detect any abnormality in either male or female partner. In the male, conventional semen analysis is now recognized to be of limited value to predict assisted reproductive treatment (ART) outcome. In contrast, sperm DNA testing has been increasingly recognised as a more promising test. Increased sperm DNA damage on male sperm is also shown to have a negative association on fertilization rates in IVF
DNA damage has been associated adversely with fertilization rates, embryo quality and pregnancy rates after ART. As there is little correlation between conventional semen parameters and sperm DNA damage, men with idiopathic infertility can have significantly high levels of sperm DNA damage which remain undetected. In this study we assessed the effect of sperm DNA on embryo quality in couples with male, female and idiopathic infertility and the impact of oocyte quality when sperm DNA damage remained constant.

**Materials and Methods:** A total of 133 couples attending for IVF were identified and of these, 89 couples (51% female, 17% male and 32% idiopathic infertility) who were having embryo transfer on day three were included in this study. Couples with failed fertilization and men with antisperm antibodies in their semen were excluded from the study. Semen analysis was performed according to WHO guidelines (1992). Native semen and sperm prepared by density gradient centrifugation were analysed for DNA damage by the alkaline Comet assay. The embryo score was calculated by multiplying embryo grade (A = 4, B = 3, C = 2 and D = 1) with the number of blastomeres for each embryo and then they were grouped into three categories low (< 12), moderate (12-24) and good (> 24) embryo grades. Similarly, sperm DNA damage was categorized into three levels of fragmentation [low (0-40%), moderate (41-70%) and high (71-100%)]. Chi square analysis was performed to evaluate the differences in embryo quality obtained from couples with male, female and idiopathic infertility. Duncan’s test for multi-group comparison was performed to analyse embryo quality within each category of DNA damage in couples with male, female and idiopathic infertility.

**Results:** A total of 678 embryos were generated, of which 53.7% were low, 27.8% were moderate and 18.5% were good quality embryos. There were no correlations between embryo quality and any conventional semen parameters. However, a significant inverse correlation was observed between embryo quality and sperm DNA damage ($r^2 = 0.131$, $P = 0.001$). When sperm DNA damage was separated into the three categories, there was a significant inverse correlation between embryo quality ($0.27 \pm 0.51$, 10.71 $\pm 0.92$ and 12.73 $\pm 1.01$) and sperm with high, moderate and low DNA damage respectively, ($P = 0.006$). High sperm DNA damage resulted in poor quality embryos (57%, 60% and 70%), in male, female and idiopathic groups of patients, respectively. Also, in the group with moderate sperm DNA damage, there was no difference in the percentage of low and moderate quality embryos in patients with male, female or idiopathic infertility. Surprisingly, couples with idiopathic infertility and low sperm DNA damage had significantly less embryos of high quality (13%, embryo grade 8.96 $\pm 1.44$) than the other two groups of patients (female infertility 28%, embryo quality 14.11 $\pm 1.49$, $P = 0.05$ and male infertility 38%, embryo quality 18.44 $\pm 2.45$, $P < 0.001$).

**Conclusion:** Sperm DNA damage impacts adversely on the quality of day three embryos in couples with male and female infertility. However, poor quality embryos in couples with idiopathic infertility and low DNA damage suggests that an oocyte defect may be a contributory aetiological factor.

We gratefully acknowledge Hamilton Thorne Biosciences, Beverly, MA 01915-6143, USA for funding the project.

**P-024 Increased body weight is associated with decreased conventional and non conventional flow cytometric sperm parameters in otherwise healthy men**

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**Introduction:** Obesity has been reported to negatively affect male reproduction. The most common aspects investigated in these patients are hormonal and sexual dysfunction, oxidative stress, and scrotal hyperthermia. As far as semenology, the studies thus far conducted have only examined the effect of obesity on conventional sperm parameters (density, motility and morphology), whereas little is known about the effects of body weight on sperm mitochondrial function, chromatin compactness and apoptosis. Therefore, this study was undertaken to evaluate conventional and non conventional sperm parameters in healthy, normal, non smoking overweight and obese men.

**Materials and Methods:** A total of 24 men with increased body weight were enrolled in this study. They were divided into two groups: 1) overweight (n = 11) with a body mass index (BMI) of 25-29.9 kg/m$^2$; and 2) obese (n = 13) with a BMI $>$ 30 kg/m$^2$, according to the WHO 1995 criteria. Normo-weight healthy non smoking men (n = 16) with a BMI of 18.5-24.9 kg/m$^2$ were recruited as controls. A complete general and andrological clinical, laboratory and instrumental diagnostic work-up was conducted on each subject. Men with systemic diseases, male accessory gland infection, history positive for cryptorchidism or varicocele, microorchidism, alcohol and/or drug abuse, endocrine diseases and recent hormonal treatment were excluded. Semen samples were collected after 3-5 days of sexual abstinence and analyzed according to the WHO 1999 criteria. The remaining spermatozoa were used for the evaluation of the following non conventional parameters, by flow cytometry: mitochondrial membrane potential (MMP), following JC-1 staining, phosphatidylserine (PS) externalization, after annexin V and propidium iodide (PI) staining, chromatin compactness, after PI staining, and DNA fragmentation, by TUNEL assay. Results were analyzed by 1-way analysis of variance (ANOVA) followed by the Duncan test. Correlation analysis was conducted by Pearson test. A statistically significant difference was accepted when the p value was lower than 0.05.

**Results:** Overweight and obese men had lower sperm density, total sperm count, progressive motility (grade a + b) and normal forms than controls. Serum LH, FSH, prolactin and testosterone levels in overweight and obese men did not differ significantly from those found in controls, whereas a significantly higher

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serum levels of 17β-estradiol and SHBG were found. Overweight and obese patients had a significantly higher percentage of spermatozoa with low MMP and 31.9 ± 9.3%, respectively) and a significantly lower percentage of alive spermatozoa (56.5 ± 6.9% and 54.1 ± 5.8%, respectively) compared to controls (low MMP = 2.8 ± 1.1%; alive = 77.8 ± 1.9%). The percentage of spermatozoa with PS externalization (10.7 ± 3.7% and 16.1 ± 3.4%, respectively), an early sign of apoptosis, or with abnormal chromatin compactness (20.0 ± 2.3% and 20.6 ± 3.1%, respectively) was significantly higher in overweight and obese patients compared to controls (PS externalization = 2.7 ± 0.5%; chromatin abnormality = 13.9 ± 0.7%). Lastly, the percentage of patients with fragmented DNA was higher in both overweight (4.1 ± 0.8%) and obese (5.6 ± 1.3%) patients compared to controls (2.3 ± 0.7%), but the difference reached the statistical significance only in the latter. Correlation analysis showed that the BMI correlated negatively with total sperm count, progressive motility, normal forms and percentage of viable spermatozoa and positively with spermatozoa with low MMP, PS externalization and DNA fragmentation, but not with chromatin compactness.

Discussion: These results showed that healthy overweight and obese men have worst conventional sperm parameters, sperm mitochondrial function and chromatin/DNA integrity. All these parameters, but chromatin abnormality, correlated significantly with BMI. The lack of andrological, systemic and/or endocrinological diseases suggest that the increased body weight has a negative impact on these parameters. Given their relevant role played on couple’s fertility, we suggest to include a body weight losing program among the therapeutic strategies of male infertility.

P-025 Effects of density gradient centrifugation and swim up techniques on sperm DNA integrity

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Introduction: A wide variety of techniques for sperm preparation are currently available, the most commonly used are density gradient centrifugation and swim up. Although these techniques appear to be effective in selecting functional sperm for assisted reproductive technologies (ART), to date, there is no consensus about which is the best method for isolating funcionally competent spermatozoa and neither about the effect of these semen processing techniques on sperm DNA integrity.

Sperm DNA damage has been linked with poor semen quality and reduced fertility. Since there is concern about the effects of DNA fragmentation in ART success and embryo quality, the evaluation of the DNA damage caused by some of the protocols of semen processing is of interest. In this study we aim to examine and compare the effect of density gradient centrifugation and swim up processing techniques on DNA integrity within populations of spermatozoa from normozoospermic and non normozoospermic subjects.

Material and Methods: Sperm preparation

One hundred and forty nine human semen samples were used in this study. Samples were obtained by masturbation after 3-5 days of sexual abstinence. After liquefaction of semen, standard semen parameters were obtained according to WHO guidelines. All of the semen samples used had a minimum concentration of 5 million spermatozoa/ml and absence of leukocytes-torospermia.

Swim up. Samples diluted in IVF plus (Vitrolife, Göteborg, Sweden) were centrifuged at 1400 rpm for 10 minutes, the sperm pellet resuspended in IVF plus and incubated for 1h at 37°C under an atmosphere of 5% CO₂ in air. After the incubation period, the entire supernatant was aspirated and analysed.

Density gradient centrifugation. Semen samples were placed on top of a 50%-90% Spermgrad (Vitrolife, Göteborg, Sweden)/IVF plus gradient, centrifuged for 20 minutes at 1200 rpm and the pellet resuspended in IVF plus. Subsequently, the swim up protocol described above was performed.

Sperm DNA fragmentation analysis

Sperm Chromatin Dispersion (SCD) test. DNA fragmentation index (DFI) and DNA degradation index (DDI) of the samples were determined by using the Halosperm® kit (Halotech DNA SL, Madrid, Spain).

2 tailed (2T) Comet assay: Single stranded (ss) and double stranded (ds) DFI of the samples were determined by using the 2T comet assay (Enciso et al. 2009).

Results: SCD test DNA damage analysis revealed that neat non normozoospermic semen samples presented a significantly (U Mann Whitney, p < 0.05) higher mean DFI and DDI than normozoospermic samples.

After semen processing with density gradient and swim up techniques, mean sperm DFI and DDI, assessed by the SCD test, were significantly reduced (U Mann Whitney, p < 0.05) in both swim up-treated and density gradient-treated spermatozoa in both groups of subjects. Similarly, the percentage of spermatozoa with ds DNA breaks assessed by the 2T comet assay, was also significantly reduced (U Mann Whitney, p < 0.05) in both swim up-treated and density gradient-treated spermatozoa in both groups of subjects. However, the percentage of spermatozoa with ss DNA breaks assessed by the 2T comet assay, was significantly (U Mann Whitney, p < 0.05) reduced only in density-gradient-treated but not in swim up-treated spermatozoa in the groups of subjects analysed.

Conclusions: The semen processing techniques swim up and density gradient centrifugation recover spermatozoa with improved DNA quality. Both methods are efficient in eliminating highly DNA damaged and double stranded DNA damaged spermatozoa but the density gradient centrifugation method is more efficient than the swim up technique in eliminating single stranded DNA damaged spermatozoa.


P-026 Can prepubertal human testicular tissue be cryopreserved by vitrification?

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Introduction: Cryopreservation of testicular tissue from prepubertal boys with cancer has emerged as an ethically acceptable strategy to preserve their fertility, since sperm banking cannot be considered in these patients. Controlled slow-freezing (SF) with dimethylsulfoxide (DMSO) as a permeating cryoprotectant (CP) has proved to be a promising approach to preserve immature human testicular biopsies. Nevertheless, an efficient cryopreservation protocol has not yet been established. Vitrification (V) might constitute a better approach by avoiding ice crystal formation and subsequent freeze injuries. Using a long-term in vitro organotypic culture system, our aim was to evaluate the efficiency of V to preserve spermatogonial cell (SPGc) survival and spermatogenic tubule (ST) integrity of prepubertal human testicular tissue, since these parameters are essential for initiation of spermatogenic processes.

Material and Methods: Testicular tissue was obtained from two patients (6 and 12 years of age) before starting gonadotoxic treatment. Controlled SF was performed with a programmable freezer using a freezing solution containing DMSO (0.7 mol/l) and sucrose (0.1 mol/l). Increased concentrations of CP and faster cooling rates were used for V. After dehydration in DMSO (2.8 mol/l) and ethylene glycol (2.8 mol/l) solution, samples were placed in open cryostaws and directly plunged into liquid nitrogen. Fresh tissue (FR), used for control purposes, and thawed and warmed biopsies were cultured for 10 days. ST integrity was evaluated by light microscopy on stained sections of tissue fixed in Bouin’s solution. SPGc survival and proliferation were evaluated by immunohistochemistry using, respectively, MAGE-A4 and Ki67 antibodies on sections of tissue fixed in formalin.

Results: Similar ST morphology was observed in both cryopreserved tissues and FR controls after long-term organotypic culture. Histological characteristics of SPGc and Sertoli cells were preserved, as well as cell-cell cohesion and cell adhesion to the basement membrane in all three groups (FR, SF and V). Pyknotic nuclei were found in the ST of FR and cryopreserved cultured tissue retrieved from the oldest boy. These cells were more frequently seen after cryopreservation, but no difference was noted between the SF and V methods. Survival of SPGc evidenced by MAGE-A4-positive immunostaining was confirmed in all cryopreserved tissue and FR controls after long-term culture. The ability of SPGc to proliferate after cryopreservation and culture was proved in all three groups by positive Ki67 immunostaining.

Conclusion: Vitrification is a convenient method for cryopreservation of immature human testicular tissue, since the process appears to be faster and cheaper than SF. As this technique preserves ST integrity and allows survival and proliferation of human SPGc in long-term organotypic culture, V might be considered as an alternative to SF, and thus a promising strategy to preserve the reproductive capacity of young boys. The functional characteristics of cryopreserved SPGc should be further evaluated through xenotransplantation experiments to determine whether SF and V are equally efficient approaches.
P-027 In-vitro addition of resveratrol in sperm cryopreservation

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Introduction: Cryopreservation of human semen is increasingly important in reproductive medicine, but it may cause damages to the spermatozoa, probably due to the production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, which may cause structural damage to biomolecules, DNA, lipids, carbohydrates and proteins, as well as other cellular components. To counteract these effects, some antioxidants such as vitamins C and E have already been used; however, the potential role of antioxidants in protecting against the damages induced by ROS during cryopreservation is still a matter of debate. While some studies indicate improvement, other has shown no effect of antioxidant treatment. Besides the routine sperm parameters, the evaluation of oxidative status could provide information to better understand the oxidative stress mechanisms aiming to evaluate the efficiency of antioxidant therapy. Therefore, the aim of this prospective study was to analyze oxidative stress markers and seminal standard parameters before and after using resveratrol, an important antioxidant, in the cryopreservation of human semen.

Material and Methods: In this prospective study, ejaculated spermatozoa from 20 infertile men and 10 fertile donors with proven fertility were examined. Individuals with azoospermia, leukocytospermia or under antioxidant supplementation were excluded from the study. Oxidative stress markers including the levels of thiobarbituric acid reactive species (TBARS; lipid peroxidation product) and the activity of the antioxidant enzymes superoxide dismutase (Sod) and catalase (Cat) were evaluated. Semen samples were divided into 4 aliquots which were treated as follows: no resveratrol, 0.1 mM, 1.0 mM, and 10.0 mM of Resveratrol. All 4 aliquots were cryopreserved and sperm motility, SOD, Cat and TBARS were evaluated both before cryopreservation and after thawing. Results were analyzed by ANOVA and Tukey’s post hoc test. Person correlation was used to calculate the relationship between response variables. Differences were considered statistically significant if p < 0.05.

Results: Cryopreservation causes a decrease in Sod activity in fertile infertile men, both with or without the use of resveratrol (p = 0.03). However, Cat activity was higher in all pos-thawing samples, both in fertile and infertile men, than before freezing the SOD activity irrespectively of its concentration in the post-thawing samples of infertile men (p = 0.03). Both fertile and infertile men showed increased TBARS values after cryopreservation, which were prevented by resveratrol, irrespective of its concentration (p = 0.03). A negative correlation was found between sperm motility and TBARS values in infertile men, both in the post-freezing (-0.868, p < 0.05) and in the post-thawing (-0.897, p < 0.05) samples.

Conclusions: The cryopreservation procedure for human spermatozoa is still far from ideal, and all improvements are important to preserve the quality of the spermatozoa stored in liquid nitrogen. Resveratrol was able to prevent post-thawing lipid peroxidation both in fertile and infertile men, reflected by the increase on TBARS and improve on antioxidant activity. However, this antioxidant was not able to prevent the motility decrease caused by cryopreservation. The present work showed that resveratrol is able to minimize lipid peroxidative damages and that it could be considered in human cryopreservation procedures, both for fertile and infertile men, at least for ICSI (Intracytoplasmic Sperm Injection), as it does not need motile sperm for achieving pregnancy.

P-028 Predictive factors of intrauterine insemination success: sperm morphology according to new suggested reference values

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Introduction: Intrauterine insemination (IUI) is widely used as a simple, inexpensive, and acceptable assisted reproductive technique. Indications include cases of cervical infertility, relative male factor infertility, anovulation, endometriosis with a healthy fallopian tube and unexplained infertility. Intrauterine insemination outcomes differ according to patient’s selection criteria, infertility factor, methods of ovarian stimulation, number of cycles performed and sperm parameters. Pregnancy rate reported is around 10 to 20% per patient, but the reported rates range from as low as 5% to as high as 70%. The number of preovulatory follicles (16mm in diameter or more) is another prognostic factor, where the presence of 3 to 4 preovulatory follicles was associated with higher pregnancy rates. Other previously discussed prognostic factors included female age and amount of motile spermatozoa after sperm preparation. On the other hand, reports have been shown that sperm morphology could not be predictive of IUI outcome. Recently, the 4th WHO data described lower reference limits for the number of morphologically normal spermatozoa. The aim of our study was to assess the likelihood of IUI success as a function of the previously described predictive factors, including sperm morphology values according to WHO (2010).

Materials and Methods: The present study included 300 couples undergoing their first Intrauterine Insemination (IUI) using father’s fresh sperm as a result of cervical infertility, relative male factor infertility, anovulation or unexplained infertility. Sperm morphology was rated according the threshold values of the WHO. All IUI cycles were preceded by ovarian superovulation with human recombinant FSH. Cycles were monitored by transvaginal ultrasound for the mean follicular volume and thickness of the endometrium. Human chorionic gonadotrophin was administered to induce ovulation, when at least one follicle measured 18mm or more. The IUI was performed with a Tom Cat catheter 36 hours after hCG injection. The luteal phase was routinely supported with micronized progesterone. Serum hCG was determined 2 weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy. The outcome measure was the occurrence of clinical pregnancy.

Results: The overall pregnancy rate (PR) was 12.8%. Women older than 35 years showed a lower PR (6.5% vs 18.2%, P = 0.017). Binary logistic regression models confirmed the significant lower chance of pregnancy occurrence for older women (OD: 0.39; IC: 0.16 – 0.96; P = 0.040). The presence of two or more preovulatory follicles on the day of hCG administration resulted in higher PR when compared to cases in which only one preovulatory follicle was present (18.6% vs 8.2%, P = 0.011). In fact, the regression model showed a more than two fold increase on probability of pregnancy when two or more preovulatory follicles were detected (OD: 2.58; IC: 1.22 – 5.46; P = 0.013). The number of inseminated motile sperm positively influences pregnancy occurrence (OD: 1.47; IC: 0.88 – 3.14; P = 0.027). However, normal sperm morphology, defined as at least 4% of normal forms, has no influence on PR (OD: 1.05; IC: 0.56 – 3.32; P = 0.936). Similar PR was observed when semen samples were classified as normal or abnormal morphology, respectively (10.6% vs 10.2%, P = 0.936).

Conclusion: In summary, our results suggest that couples with the best probability of pregnancy are those in which the woman is under 35 and the inseminated motile sperm count was at least 1 million. In addition, the ideal stimulation cycle allows the recruitment of at least two follicles measuring more than 16mm on the day of hCG administration. Our results demonstrate that sperm morphology normalcy, according to the new reference value, has no predictive value on IUI outcomes. We could suggest that, considering the above mentioned required parameters, the success probability could not be influenced by altered sperm morphology normalcy value.

P-029 Molecular characterization of the CAG polymorphism and mutations of the androgen receptor genes in fertile and infertile agriculture men

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Introduction: Semen analysis is the cornerstone evaluation of the infertile men. Male phenotype, as well as the beginning of the spermatogenesis are intrinsically
linked to cellular events responsive to androgens. The hormonal action of the two most important androgens (testosterone and 5α-dihydrotestosterone) is mediated by the action of the androgen receptor (AR). In fact, the AR is a nuclear transcription factor code by the AR genes. The aim of this study was to molecularly characterize the mutations and the CAG polymorphisms in a population of infertile men who lives in a rural region, with the diagnosis of idiopathic infertility.

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

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

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

P-030 Varicocelectomy impact on assisted reproductive approach
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**Introduction:** Male factor is responsible itself for 20% of the total infertility cases, contributing to other 30-40% of the cases. Varicocelectomy plays an important role in this scenario, affecting 40% of infertile men. It is also present in approximately 10-20% of normal male population. Varicocelectomy has been considered the infertility treatment of choice since 1952, when the first spontaneous post-varicocelectomy pregnancy was reported. The Practice Committee of the American Society for Reproductive Medicine indicates surgical repair of varicocele when an adult has a palpable varicocele and is unable to achieve pregnancy in subsequent attempts, or has an abnormal semen analyses or desires future fertility. However, even when varicocele is repaired and all beneficial effects over semen quality appears to be obtained, unassisted pregnancy rates vary from 19% to 35%. Therefore, assisted reproductive technologies (ART) are important for the management of couples with male factor infertility associated with varicocele, even in cases of men who underwent varicocelectomy. The aim of this study was to evaluate the contribution of varicocelectomy, proposed before infertility treatment, in improving the sperm quality and consequently the outcomes of ART.

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

**Results:** No significant differences were found between the two groups regarding demography and cycle’s general characteristics. Similar maternal age (33.0 ± 4.6 vs. 33.8 ± 3.8, P = 0.1872), total dose of recombinant follicle stimulating hormone (FSH) administered (2371 ± 785 vs 2249 ± 640, P = 0.2943), estradiol (E2) levels on the day of hCG administration (1926 ± 364 vs 2002 ± 201, P = 0.8490), number of aspirated follicles (17.3 ± 12.5 vs 16.4 ± 10.5, P = 0.9752), oocyte yield (75.4% vs 77.6%, P = 0.6401), and metaphase II (MII) oocyte rate (61.2% vs 66.4%, P = 0.6217) were observed. However, our results indicate that paternal age (36.1 ± 5.5 vs 37.8 ± 4.7, P = 0.0319) and period of infertility (2.7 ± 0.3 vs 6.0 ± 4.9, P < 0.001) were significantly higher in men who underwent varicocelectomy. Although semen volume was higher in Group 1 (3.3 ± 0.3 vs 2.5 ± 0.14, P = 0.0043), sperm concentration (30.08 ± 4.01 vs 24.1 ± 2.42; P = 0.138), progressive motility (38.2 ± 26.9 vs 38.7 ± 20.8; P = 0.881), as well as normal morphology (2.6 ± 0.44 vs 2.4 ± 0.37; P = 0.7292) were similar between groups. Fertilization rate was significantly higher in Group 1 (73.2% vs 64.9%, P = 0.0377). However, no differences were observed on pregnancy (31.1% vs 30.9%; P = 0.9806), implantation (22.1% vs 17.3%; P = 0.5882) and miscarriage rates (21.7% vs 23.9%; P = 0.8401) in Groups 1 and 2.

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

P-031 Seminal vesicle ultrasonographic characterization in infertile patients with type 2 diabetes mellitus
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**Introduction:** Ejaculatory dysfunction is common in type 2 diabetes mellitus (DM) and it is a potential cause of infertility in these patients. The lack of studies exploring the ultrasonographic characteristics of the seminal vesicles (SVs) in diabetic patients and the high frequency of neuropathy in these patients, which also affects SV function, prompted us to evaluate the ultrasonographic characteristics of the SVs in infertile patients with DM. To accomplish this, prostate-vesicular transrectal ultrasonography (TRUS) was performed in infertile patients with type 2 DM without any known cause of infertility. Patients with idiopathic infertility and infertility plus male accessory gland infection (MAGI) were selected a control and a well-defined clinical model of SV causes of infertility.

**Methods:** A total of 75 infertile patients were selected for this study according to the following criteria: 1) Control group: 25 infertile patients without DM and no known causes of infertility. They had a mean ± SEM age of 35 ± 0.5 years (range 28-37, years); 2) MAGI group: 25 infertile patients without DM but with MAGI, diagnosed according to the WHO criteria (1993). They had a mean ± SEM age of 31 ± 0.6 years (range 27-36, years); 3) DM group: 25 infertile patients with type 2 DM and no other known causes of sperm parameter abnormalities. They had a mean ± SEM age of 34 ± 0.8 years (range 28-36,
year). All patients underwent TRUS examination after 1 day of sexual abstinence, before and after 1 hour from ejaculation. The prostate-vesiculare region was assessed using a transrectal 7.5 MHz biplan biconvex transducer (Esaeote GPX Megas, Genova, Italy) and the following SV ultrasound parameters were recorded from each patient, by the same operator: 1) body antero-posterior diameter (ADP); 2) fundus APD; 3) parietal thickness of the right and left SVs; 4) number of polycyclic areas within both SVs; 5) fundus/body ratio; 6) difference of the parietal thickness between the right and the left SV; and 7) pre- and post-ejaculatory APD difference.

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

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

P-032 Identification and functional characterization of a point mutation in the gene encoding the sperm factor phospholipase Cζ in an infertile male
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Introduction: Mammalian oocytes are thought to be activated by a sperm-specific phospholipase Cζ, PLCζ. Whilst some types of human male infertility appear to be caused by failure of the sperm to activate the oocyte, it remains unclear as to whether this is due to defective PLCζ. Here, we describe the identification of a point mutation in the catalytic region of the PLCζ gene in one infertile male diagnosed with oocyte activation deficiency. The consequence of this mutation upon functional ability were investigated using mammalian cell expression and mouse oocyte injection.

Materials and Methods: PLCζ exons were amplified by polymerase chain reaction from fertile/infertile males, sequenced, and compared with PLCζ wild-type (WT) and cDNA sequences. Constructs were engineered in which WT and mutant (M) PLCζ were fused with a fluorescent mCherry tag. An additional construct was created in which the C-terminal region of PLCζ (partial Y and entire C2 domain) had been intentionally deleted (∆C2). In order to assess oocyte activation and calcium (Ca2+) release ability, WT- and M-PLCζ cRNA were microinjected into mouse oocytes. We also assessed HEK293T cells transfected with WT-, M-, and ∆C2-PLCζ for their ability to respond to ATP, a potent agonist of Ca2+ release. The expression and localization of the three PLCζ isoforms were studied in HEK293T cells using fluorescent and confocal microscopy. DAPI and Wheat Germ Agglutinin-GFP conjugate were used as markers of the nucleus and plasma membrane, respectively.

Results: DNA analysis revealed a point mutation (His→Pro) within the catalytic domain of PLCζ in one infertile male with normal sperm morphology. Computer modelling indicated significant effect upon protein structure and ability to interact with other proteins. Whilst all mouse oocytes injected with WT-PLCζ cRNA exhibited Ca2+ oscillations characteristic of fertilization, those injected with M-PLCζ cRNA failed to respond. Live cell imaging of HEK293T cells demonstrated that the Ca2+ response for cells expressing WT-PLCζ was significantly diminished compared to that of M- and ∆C2-PLCζ, resembling the Ca2+ response observed in cells transfected with constructs absent of PLCζ. Mean amplitudes were significantly different at 5μM (WT: 1.6, M: 2.8, ∆C2: 3.1), and 100μM ATP (WT: 1.9, M: 3.6, ∆C2: 3.2). The percentage of responding cells was significantly lower at 5μM (WT: 26%, M: 83%, ∆C2: 88%) and 100μM ATP (WT: 21%, M: 86%, ∆C2: 91%). Fluorescent and confocal imaging of transfected HEK293T cells demonstrated that the three constructs exhibited cytoplasmic localization with clear differences in expression levels (WT > M > ∆C2-PLCζ).

Conclusions: Our study identified, for the first time, a single PLCζ point mutation (His→Pro) in an infertile male diagnosed with oocyte activation deficiency. Computer modeling suggested significantly impaired 3D structure and ability to interact with other proteins. Concomitantly, M-PLCζ cRNA was unable to cause Ca2+ release, or induce activation upon injection into mouse oocytes. HEK293T cells transfected with M-PLCζ revealed reduced levels of expression when compared to those transfected with WT-PLCζ. Cells transfected with the ∆C2-PLCζ construct expressed levels of mCherry fluorescence which were significantly lower than the WT- or M-PLCζ constructs. Further experiments demonstrated that HEK293T cells expressing WT-PLCζ failed to release Ca2+ when stimulated with ATP, indicating that over-expression of active WT-PLCζ may have exhausted internal Ca2+ stores, unlike the M- or ∆C2-PLCζ constructs. Our studies indicate that defective forms of PLCζ may play an important role in certain types of male factor infertility and suggests that an active recombinant PLCζ may represent a valuable therapeutic tool for such patients.

P-033 A comprehensive mutation screen of genes involved in sperm motility
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Introduction: Adequate motility is crucial for the fertilizing potential of sperm. Impaired motility of human sperm cells, i.e. asthenozoospermia, is detected in ~20% of couples presenting with infertility. Although genetic causes are thought to underlie asthenozoospermia, none have been identified so far. Mouse knockout models and in vitro functional experiments have put forward a number of candidate genes for asthenozoospermia in humans.

Material and Methods: We performed a case-control study and screened 30 men suffering from isolated asthenozoospermia and 90 controls for mutations in the coding regions of nine asthenozoospermia candidate genes: AKAP4, CATSPER1, CATSPER2, CATSPER3, CATSPER4, GAPDH5, PLAG26, ADCY10 and SLCA10. Whenever mutations were found, their putative impact was evaluated in silico. To account for a possible effect of heterozygous mutations, we assessed imprinting of all candidate genes by studying the expression pattern of heterozygous SNPs in testis biopsies of five unrelated men.

Results: We identified 10 asthenozoospermia-specific mutations in AKAP4 (n = 1), CATSPER1-4 (n = 6), ADYC10 (n = 2) and PLAG26 (n = 1). These mutations were distributed over six patients, with one patient carrying three mutations, two patients carrying two mutations each and three patients having a single mutation. None of these mutations were found in controls. In silico analysis showed that eight of the mutations had a negative BLOSUM score, were located in conserved residues and/or were located in a functional domain. All mutations were heterozygous and expression analysis demonstrated that CATSPER 1 and 4 are imprinted.

Conclusions: Given their putative effect on protein structure, their location in conserved sequences and/or functional domains, and their absence in controls, the identified mutations in AKAP4, CATSPER2, CATSPER3, CATSPER4,
P-034 The impact of hyaluronic acid binding sperm selection plus MSOME to improve pregnancy rates in male factor
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Introduction: implantation and pregnancy after ICSI is positive correlated with morphological normality of spermatozoon. Sperm with a morphologically abnormal nucleus usually have low fertility potential. The MSOME (Motile sperm organelle morphology examination) method let to show the morphological normality of sperm cell organelles observed at high magnification, but is time consuming. Spermatozoon have a specific affinity to bind HA (hyaluronic acid) a natural protein found in the cumulus complex surrounding the oocyte. The binding reflects sperm maturity and functionality, due to a relationship between sperm receptors for zona pellucida and the HA, therefore a normal sperm have higher chance to bind HA. The aim of this study was to compare the pregnancy, implantation and miscarriages rates when we combined two sperm selection techniques: HA binding test and MSOME before ICSI versus conventional ICSI.

Materials and Methods: retrospective and descriptive study from august 2009 to January 2020 of 40 patients with female ages between 30 and 38 years old, with severe male factor who were candidates to ICSI. We divided the patients in two groups: 20 patients for Group I (HA plus MSOME prior ICSI) and 20 patients for Group II (Conventional ICSI). The sperm sample in Group I was prepared by gradients and adjusted to 4000 motile high density sperm cells, obtained from the pellet fraction and were transferred to a 5 ml observation microdroplet containing HA binding solution, after 15 minutes, the leftover spermatozoa that have not been bind to HA were removed and washing with HTF medium with HEPES. Between 10 to 15 HA binding spermatozoa were chosen and transfer to an observation microdroplet with PVP solution on a glass-bottomed dish under sterile paraffin oil and used for individual selection by MSOME, after that we did ICSI procedure. In group II we process the sperm sample with standard ICSI technique. Mann-Whitney U-tests were carried out. Significance was defined as p < 0.05. The statistical analysis was performed using SPSS Software.

Results: a total of 82 embryos were transferred. The media of embryos transferred were 2.05 per patient. Pregnancy, implantation and miscarriage rates were: 65.00 %, 26.82% and 18.18% in Group I versus 47.00%, 15.78 % and 33.33% in group II respectively. This preliminary results shows statistical differences between both groups in favor of group I.

Conclusions: the combination of HA binding test plus MSOME seems to improve success rates for Reproductive Assisted Techniques in male factor. One disadvantage of the MSOME is that is time consuming. These results suggest that morphologically normal sperm have higher chance to bind HA and therefore MSOME selection of spermatozoon is probably more easy and faster. These two tests for normal morphologically sperm selection will be incorporated in our daily routine before the ICSI procedure. To confirm the effect of HA sperm selection and MSOME on the ICSI outcome further studies are required.

P-035 Analysis of the clinical outcomes of rescue ICSI after total fertilization failure in 115 conventional IVF cycles
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Introduction: To evaluate the outcome of the rescue ICSI after total fertilization failure in 115 IVF cycles.

Material and Methods: 8359 in vitro fertilization (IVF) cycles from January 1999 to May 2009 were analyzed retrospectively. The reasons of IVF cycles were mainly with tubal factor infertility, endometrosis and mild oligospermia. Ovarian down-regulation was achieved by either a long leuprolide acetate protocol or short protocol. The women underwent controlled hyperstimulation with recombinant FSH(r-FSH, Gonal-F). Ovarian response was daily monitored by ultrasound. The dose of r-FSH was individually adjusted according to the ovarian response. When one or more follicles reached 18 mm in diameter, 10000 IU hCG were administered. Oocyte retrieval was performed 34 to 36 hours after hCG administration. And conventional IVF was carried out. All patients scheduled for embryo transfer (ET) received intramuscular progesterone (40mg/day for 17 days) or hCG (2000 IU every 3 days for 4 times). We conducted polar body observation following overnight sperm-oocyte coinubcation for 14 to 16 hours. We did rescue ICSI for most IVF cycles of total fertilization failure. We retrospectively analyze the clinical outcome of rescue ICSI cycles from January 2004 to May 2009. The total fertilization failure rate was calculated. The prognosis for clinical pregnancy and delivery after total fertilization failure were analyzed.

Results: 8359 cycles were carried out with conventional IVF from January 1999 to May 2009. 232 cycles were total fertilization failure. The overall fertilization failure rate was 2.78% (232/8359). From January 2004 to May 2009, 838 MII oocytes in 115 cycles received rescue ICSI. The rate of normal fertilization and cleavage were 71% and 93.78%, respectively. On the day of embryo transfer, the good quality embryos rate was 51.79%. 100 cycles(100/115, 86.89%)had embryo transfer. The average transfer embryos were 2.30 ± 0.67 (1 to 3). Three patients froze all embryos due to ovarian hyperstimulation syndrome. The biochemical pregnancy rate was 11%, and five babies were born. One case was early miscarriage and one case was ectopic pregnancy.

Conclusions: Rescue ICSI can achieve high rate of fertilization and cleavage, and produce variable embryos and pregnancies, but it can not improve the pregnancy results.

P-036 Sperm aneuploidy and meiotic recombination in patients with post-vasectomy obstructive azoospermia
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Introduction: Many studies have been published concerning the cytogenetic analysis of ejaculated and testicular sperm from infertile males. However little is known about meiotic recombination, one of the crucial events that control the pairing of homologous chromosomes for their proper segregation. The purpose of this study was to analyse sperm aneuploidy rates and meiotic recombination in testicular biopsies of post-vasectomy obstructive azoospermia (OA) patients.

Material and Methods: This study includes eight post-vasectomized patients attending an IVF setting for infertility treatment (January 2008-December 2009). Mean patients age was 47.8 ± 6.9 years and mean years after vasectomy were 14.0 ± 5.9. Sperm aneuploidy and diploidy were evaluated using fluorescence “in situ” hybridisation (FISH) for chromosomes 13, 18, 21, X and Y (Vysis, Downers Grove, IL, USA). The incidence of disomic and diploid sperm were compared with ejaculated sperm samples from a control group formed of 10 normozoospermic fertile donors. Immunocyto genetics in pachytene stage spermatocytes (prophase I) was performed using the following primary monoclonal antibodies: SC3P (Sypnatonemal Complex Protein 3), purified rabbit antiserum for the axial/lateral elements of the synaptone mal complex (SC) (Novus Biologicals, Littleton, CO, USA); CREST human anti-centromere protein for the centromeric regions (Fisher Scientific, Park Lane, Pittsburgh, PA, USA) and MLH1 (mismatch repair protein MutL homolog 1) purified mouse anti-MLH1 monoclonal antibody that co-localizes to sites of meiotic cross-over (BD Pharmingen, San Jose, CA, USA). MLH1 foci were scored to assess total recombination levels. Percentages of pachytene cells with 0, 1, 2, 3, 4, 5 or 6 recombination foci or exchanges (E0, E1, E2, E3, E4, E5 or E6) were also evaluated. Chi-square test was employed to compare the incidence of sperm chromosomal abnormalities between the study group and controls.
Results: A total of 31,510 testicular sperm cells were evaluated for chromosomes 18, X and Y, and 21,162 for chromosomes 13 and 21. There were no statistical differences compared to the control group in the percentages of disomies for sex chromosomes (0.18% vs. 0.21%), chromosome 13 (0.07% vs. 0.07%), chromosome 18 (0.003% vs. 0.03%) and chromosome 21 (0.09% vs. 0.12%). Total diploidy rates were also similar between groups (0.05 vs. 0.10).

In the immunocytogenetic study, the mean number of pachyteny cells scored per patient was 27.3 ± 17.5. Total mean recombination level was 47.8 ± 2.2 MLH1 foci, with the following distribution of exchanges: E0 = 0.5%, E1 = 15.3%, E2 = 55.2%, E3 = 22.9%, E4 = 5.7%, E5 = 0.4% and E6 = 0.02%. All of the patients followed a similar pattern except one, an outlier patient with two-times standard deviation from the mean MLH1 foci of the group (43.7 ± 2.4 and 47.8 ± 2.4, respectively). This patient showed a percentage of pachyteny cells with ≥ 3 exchanges half-decreased (16.4%) compared to the total of the group (29.0%).

Conclusions: Testicular sperm from post-vasectomy patients did not show differences in aneuploidy rates compared to ejaculated sperm from normozoospermic fertile men. Recombination levels showed a similar pattern in all cases except one and were comparable to the values previously described by other authors. Inter-individual differences have been also reported in different studies.

P-037 Decreased meiotic recombination and higher risk of sperm aneuploidy in non-obstructive azoospermia patients
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Introduction: During the first meiotic division in spermatogenesis there are two critical events: synopsis and recombination. A mistake in one of these two processes should produce incorrect segregation of chromosomes. Several studies have suggested that errors in recombination are a major cause of aneuploidy in gametes. The aim of this study was to assess the correlation between meiotic recombination and the incidence of chromosomal abnormalities in testicular spermatooza from azoospermic infertile males.

Material and Methods: From January 2008-December 2009, meiotic recombination in pachytene stage cells and sperm aneuploidy were evaluated in samples obtained from testicular biopsies of 7 non-obstructive azoospermic patients (NOA). The study group was compared with a control group formed of 8 post-vasectomy patients (OA). Mean age for NOA patients was 35.4±4.8 years (range: 30-45), with mean FSH = 10.5 mUI/mL (range: 2-29), mean LH = 4.68 ± 2.6 mUI/mL (range: 0.21-8) and mean Testosterone = 12.31 ± 1.24 nmol/L (range: 2-29). Mean age in the OA control group was 47.8 ± 6.9 years (range: 38-60). Meiotic recombination was assessed using immunocytogenetics with three primary monoclonal antibodies: SCP3 directed to axial/lateral elements of the synaptonemal complex (Novus Biologicals, Littleton, CO, USA); CREST directed to the centromeres of chromosomes (Fisher Scientific, Park Lane, Pittsburgh, PA, USA) and a MutL homolog I antibody (MLH1), which is a mismatch repair protein that co-localizes to sites of meiotic crossovers (BD Pharmingen, San Jose, CA, USA). Aneuploidy rates on sperm for chromosomes 13, 18, 21, X and Y were analyzed by fluorescence “in situ” hybridization (FISH) (Vysis, Downers Grove, IL, USA). Statistical analysis was done using Chi-square test, t-Welch test and t-test appropriately in each case.

Results: A total of 74 pachytene cells in NOA patients and 218 in the OA control group were analysed. The overall mean number of MLH1 foci per cell was 29.0%.

Conclusions: Our study shows a decrease in recombination levels and increased sperm aneuploidy rates in NOA patients. These findings would corroborate the correlation between both parameters, since the only two patients with normal recombination had the lowest aneuploidy rates.

P-038 Evaluation of the effects of different in vitro incubation conditions on sperm DNA integrity
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Introduction: Prolonged in vitro incubation of spermatozoa has been shown to have adverse effects on sperm motility, vitality as well as on DNA integrity. Knowledge regarding how shorter incubation periods prior to the IVF/ICSI procedure affect semen quality is however limited. The aim of the present study was to examine if sperm DNA integrity was affected during incubation in three different conditions for 2 hours after sperm preparation prior to the IVF/ICSI procedure.

Materials and Methods: Density gradient centrifuged samples from two hundred men undergoing infertility work-up were included in the study. Following gradient centrifugation one reference sample was frozen immediately. Thereafter samples were divided into three aliquots and incubated for two hours in either1) room temperature (23-24 °C); 2) in a 37°C humidified incubator with 6%CO2 and 5%O2 or 3) in a 37°C humidified incubator with atmospheric air. The Sperm Chromatin Structure Assay (SCSA) was used to assess the extent of sperm DNA damage. Sperm DNA fragmentation was expressed as DNA fragmentation index (DFI).

Results: A statistically significant increase in DFI was seen in density gradient prepared samples incubated for 2 hours at 37°C, 6%CO2 and 5%O2 compared to the reference sample taken immediately after preparation. This was the case also for samples incubated at 37°C in atmospheric air. Moreover, statistically significant lower DFI levels were seen in the group incubated at room temperature compared to those incubated at 37°C, 6%CO2 and 5%O2 or at 37°C in atmospheric air.

Conclusions: In order to prevent further sperm DNA damage after density gradient preparation prior to the IVF/ICSI procedure, spermatozoa should be stored at room temperature.

P-039 The correlation between total antioxidant capacity and nitric oxide concentrations in seminal plasma with sperm DNA damage in infertile males
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Nitric oxide (NO) is a free radical which produced by most of cells and tissues in the body and mediates a number of biological functions. NO in low levels involves in a Variety of male reproductive processes such as spermatogenesis, spermiogenesis, sperm motion, sperm metabolism and sperm capacitation. But high levels of NO have direct detrimental effects on sperm parameters.

Seminal plasma has antioxidant defense mechanisms to protect sperma-tozoa against free radicals. These antioxidants defense mechanisms limit the production of free radicals, scavenge them, and repair cell damage. Also there is a complex interaction between the pro-oxidants and antioxidants that results in the maintenance of intracellular homeostasis.

The aim of this study was to determine the concentrations of the nitric oxide and total antioxidant capacity (TAC) in seminal plasma and their correlation with sperm DNA damage in infertile and fertile males.

Materials and Methods: Semen samples from 45 infertile men and 70 normozoospermic men were examined for DNA damage, nitric oxide concentration and TAC. DNA damage was measured by comet assay, nitric oxide concentration was evaluated by Griess assay and TAC was measured in seminal plasma.
Introduction: Since spermatozoa movement requires great amount of energy defects in mitochondrial respiratory function is assumed to cause a decline in mobility and, consequently, decrease of fertility. The A3243G transition is the most extensively investigated tRNA gene mutation produces a severe combined respiratory chain defect, with almost complete lack of assembly of complex I, IV, and V, and a slight decrease of assembled complex III. This assembly defect occurs despite a modest reduction in the overall rate of mitochondrial protein synthesis.

Materials and Methods: We collected 200 semen samples from 200 infertile men with asthenozoospermia (a < 25% or a + b < 50%; WHO 1999). The samples with sperm agglutination, high viscosity and increased numbers of seminal leukocytes (leukospermia) were not taken into consideration. Blood samples obtained from patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS syndrome) were used as control of restriction endonuclease activity.

DNA was extracted from each semen sample. The A3243G transition was detected by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis with endonuclease BsuRI.

Results: The A3243G transition was excluded in all extremely variable (sperm motility (a + b) (3%–38%) semen samples.

Conclusions: It seems that biochemical defects in mitochondrial respiration and oxidative phosphorylation caused by the A3243G mutation in the mitochondrial DNA don’t affect the sperm motility.

P-042 Different transcriptome is found between sperm samples (ss) from patients undergoing in vitro fertilization (ivf) achieving or not pregnancy

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Introduction: Basic sperm analysis is providing limited information to explain and predict male infertility and pregnancy failures in assisted reproduction techniques (ARTs) mainly because of the lack of information regarding the molecular causes (i.e.: mRNA presence or DNA oxidation). New tools are trying got be developed to improve sperm analysis. To this end, massive testing equipments such as microarrays are interesting options to be employed in assisted reproduction. Our aim with this work is to use microarray technology to characterize differential gene expression profiles between SS from infertile patients (IP) achieving or not pregnancy in IVF cycles with donated oocytes from young healthy women. We used ovoidonum program in this study in order to avoid any female factor bias.

Material and Methods: Cases and controls study with 10 SS obtained from IP undergoing IVF cycle with donated oocytes. After freezing aliquots of the SS employed for IVF cycle from patients accomplishing the inclusion criteria, we identified samples from which pregnancy was achieved (P, n = 5) or not (NP, n = 5). Sperm mRNA was extracted using Trizol protocol, suspended in DEPC-treated water and frozen at -80°C until the microarray experiments were performed. RNAs were analyzed on Agilent Bioanalyzer 2100. The results were evaluated to detect those genes differentially expressed (GDE) (Three criteria were used to define GDE in the different sample sets: genes that were common in groups (P and NP), showing an absolute fold change expression of 2.0 times or more and a corresponding fold change p-value lower than 0.05) and exclusive genes (EG) expressed in one group but not in the other (Two criteria were used to define EG: spot intensity level greater than the mean of the density of the negative control plus 2 times standard deviation in one of the two groups. In our experiments this mean intensity was 5.85 units).

Results: The total number of expressed genes (TNEG) detected in our microarray analysis was 14811. 12351 (83.4% of TNEG) genes were commonly expressed in both groups (P and NP). Among those common transcripts, 94 (0.72% of TNEG) GDE were found in group P presenting increased expression and being the most differentially expressed the Homo sapiens chromosome 10 open reading frame 119 (C10orf119) (NM_024834) with a fold change of 34.27. However, 68 GDE (0.55% of TNEG) were found in group NP, being the most differentially expressed the Homo sapiens phospholipase A2, group IIa (platelets, synovial fluid) (PLA2G2A) (NM_000300) with a fold change of 42.31. Furthermore we detected 1219 (8.2% of TNEG) EG in group P presenting the highest spot level intensity, 9.27 units, the Homo sapiens matrix metallopeptidase 12 (macrophage elastase) (MMP12) (NM_002426) and 1241 (8.37% of TNEG) GDE in group NP presenting the highest spot level intensity, 8.37 units, the Homo sapiens chromosome 10 open reading frame 64 (BC034937).
Conclusions: This work reveals profound differences between expression profiles between samples which achieved pregnancy vs. those unable in IVF. These differences could be potentially employed to detect IVF success markers, encouraging us to further address the molecular basis of sperm physiology and improve diagnostic accuracy of sperm analysis.

Materials and Methods: A total of 24 semen samples was collected from male partners (mean age 39 years old) of couples who have undertaken an assisted reproduction protocol in our center at Cervlesi Hospital in Catelloca (Italy).

After collection all samples were allowed to liquefy at 37°C for 30 minutes and after analysed and processed for high performance liquid chromatography (HPLC): concentration, motility and morphology, were evaluated according to standards set by the World Health Organization (WHO).

The analysis of AA levels was done using a traditional method of HPLC with UV detection: sample were centrifuged at 3000 rpm for 5 min, 100 μl of the supernatant was removed, added to 900 μl of cold methanol and centrifuged at 3000 rpm for 10 min. The supernatant was stored at -20°C until analyzed by HPLC. The statistical analysis was carried out using the t-test and probability values of < 0.05 were accepted. Correlations were calculated using linear regression analysis.

Results: The highest AA value obtained is 425 m M. the lowest is 92 m M. Semen samples showing a normal concentration, morphology and motility were characterized by high levels of AA, on the contrary samples with subnormal parameters of seminal fluid show lower levels of AA.

In according to these data is possible to point out a correlation between motility and AA concentration (p < 0.0001; r = 0.96), morphology and AA concentration (p < 0.0003; r = 0.72), sperm count and AA concentration (p < 0.003; r = 0.63).

Conclusions: The presence of ascorbic acid in seminal plasma affects significantly on sperm’s motility, morphology and, in lesser extent, on concentration.

In fact seminal motility and morphology decreased proportionally with AA levels present in the seminal fluid. Several studies supported these correlations demonstrating that a decrease of an important antioxidant levels, like the ascorbic acid, and the correlated increase of ROS, leads to sperm membrane lipid peroxidation and sperm DNA damage. Determination of seminal AA levels in men with idiopathic infertility should become a routine examination in future because it can be useful in fertility assessment and in therapeutic protocols who use the supplementation of antioxidants.

Our intention for the future is to support our thesis with more data and to combine the analysis of AA levels with another parameter as Malondialdehyde (MDA) concentration to assess lipid peroxidation on sperm membrane.

P-045 Autocrine regulation of human sperm motility by met-enkephalin
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Introduction: Endogenous opioid peptides participate in the regulation of reproductive physiology at multiple sites and appear to be increasingly important in sperm cell function. Enkephalins are opioid peptides present in human semen which are involved in the regulation of sperm physiology. Specifically, met-enkephalin is synthesized by proteolysis of their precursor por-enkephalin (PENK) and its effect is inactivated by enzymes which are present in sperm cells. Although spermatogenic cells are a major site of precursor RNA expression, PENK is not efficiently translated in mouse testis. In spite of that, some of PENK-derived peptides are stored in the sperm acrosome but the role of its products is not clearly in mature spermatozoon. Therefore, the aim of this study was to study the presence of PENK and met-enkephalin in human spermatozoon and to clarify the effects of endogenous met-enkephalin on sperm motility.

Materials and Methods: The expression and localization of PENK and met-enkephalin was analyzed by RT-PCR and immunofluorescence techniques. Met-enkephalin secretion was analyzed by immunofluorescence techniques. Isolated spermatozoon by Percoll gradient were co-incubated with specific enkephalin-degrading enzyme inhibitors (lehistina en tiophran) and the opioid receptor antagonist naloxone. The motility analysis was conducted by computer-assisted sperm analysis (SCA) at 0, 0.5, 1 and 3 hours of incubation.

Results: We found transcript of PENK in mature spermatozoon and its protein was localized in the equatorial/post-acrosomal region. Met-enkephalin was localized in acrosomal region of the sperm head, but in 10% of the cases, we not observed immunoreactivity in this region. Moreover, met-enkephalin immunoreactivity decreased during the time. The inhibition of enkephalin-degrading enzymes in isolated spermatozoon increased the sperm motility during the time, being this effect reversed by naloxone.
Conclusions: Met-enkephalin and its protein precursor PENK are present in human sperm cells; therefore, spermatozoa may be generated met-enkephalin de novo by proteolysis of PENK. Met-enkephalin is secreted by sperm cells and it may be involved in the control of sperm motility by auotocrine mechanisms. In conclusion, knowing the mechanism of secretion of met-enkephalin and other opioid peptides from spermatozoa may be useful for define functional state of cell sperm.

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P-046 IUI outcome is not affected adversely by prolonged incubation of washed sperm at room temperature until IUI is performed

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Introduction: It is known that prolonged exposure of sperm to seminal plasma is harmful to sperm motility. Also, excessive incubation of washed sperm in media prepared for intrauterine insemination or in vitro fertilization could be a factor of hyperactivation, one proposed marker for capacitation. Even though the processed sperm has good quality, not all women inseminated could expect positive outcome. We hypothesized that the pregnancy rate of IUI would be affected by prolonged exposure of processed sperm to media. Thus the objective of our study is to determine the correlation between time intervals from the end of sperm wash to intrauterine insemination and IUI outcome.

Design: retrospective study

Materials and Methods: 192 IUI treatment cycles performed from June 2009 to December 2009 were analyzed. Women received 50–150mg of clomiphene citrate(cc) daily for consecutive days. Follicular development was monitored by trans-vaginal ultrasound. In 34–38 hours after administration of hCG, a single IUI was performed by physician. Semen specimens were produced either by trans-vaginal ultrasound. In 34–38 hours after administration of hCG, a single IUI was performed by physician. Semen specimens were produced either at our clinic or at home. After liquefaction, sperm wash was performed by a density gradient centrifugation. Investigator recoded time start of sperm wash and time finish of sperm wash. Processed sperm was placed at room temperature until IUI was performed. Time of IUI was also recorded by physician. Our study population is divided into two groups based on the time intervals from the end of sperm wash to IUI.

- group A: ≤ 30 minutes, n = 74 (n: number of women performed IUI)
- group B: >30 minutes, n = 118 (n: number of women performed IUI)

Results: Female ages ranged 27–48 years with a median of 37 years. There was no difference between Group A and B including female ages, indication, methods of sperm processing, processed sperm. Time intervals from the end of sperm wash to IUI ranged 2–194 minutes with the mean 43 minutes. Total IUI pregnancy rate was 16.7% (32/196), pregnancy rates have no significant difference between group A (15/74, 20%) and group B (17/118, 14%) but it shows pregnancy rate was 16.7% (32/102). Pregnancy rates have no significant difference between group A and B and indication, method of sperm processing, processed sperm. Time of IUI was also recorded by physician. Our study population is divided into two groups based on the time intervals from the end of sperm wash to IUI.

Conclusion: Our result shows that the pregnancy rate is slightly declined in group B. This result is supported by fertility ability of sperm decreases when sperm exposes to media excessively. We supposed two factors influence to fertility ability of sperm. First, prolonged incubation of sperms in media adversely affects motility of sperm by exhaustion of energy sources in medium by spermatozoa. Second, present premature capacitation including hyperactivation is a negative factor for fertility ability because hyperactivated sperm cannot traverse the uterotubal junction. Premature capacitation of washed sperm may occur due to removing of seminal plasma. Meanwhile sperm incubation at room temperature until IUI was performed. Time of IUI was also recorded by physician. Our study population is divided into two groups based on the time intervals from the end of sperm wash to IUI.

We recommend IUI should be performed as soon as sperm processing is completed, otherwise sperms should be placed at room temperature until IUI is performed.

P-047 Maternal use of acetaminophen, ibuprofen and acetylsalicylic acid during pregnancy and cryptorchidism: a population-based cohort study

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Introduction: The cyclooxygenase (COX) inhibitors, acetaminophen, ibuprofen and acetylsalicylic acid have endocrine disruptive properties in the rainbow trout. In man, aspirin blocks the androgen response to human Chorionic Gonadotropin (hCG). Since hCG-stimulated androgen production in uroto is crucial to normal testicular descent, exposure to COX inhibitors at vulnerable times during gestation may interfere with normal testicular descent. We thus examined whether prenatal exposure to acetaminophen, ibuprofen and acetylsalicylic acid was associated with increased risk of cryptorchidism.

Material and Methods: Our study made use of data on 48,397 live-born boys of mothers enrolled in the Danish National Birth Cohort during 1996-2002. During these boys’ early childhood, 1,002 cases of cryptorchidism were identified and of these, 577 boys were verified as having undergone orchiopey. Mternal use of acetaminophen, ibuprofen and acetylsalicylic acid was assessed in 4 computer-assisted telephone interviews and one self-administered enrollment questionnaire. Adjusted hazard ratios (HR) of cryptorchidism were estimated by Cox regression analysis.

Results: Exposure to acetaminophen in both the 1st and 2nd trimesters was associated with increased risk of cryptorchidism (HR = 1.39, 95% confidence interval (CI): 1.06 - 1.83). Cumulative exposure during more than 4 weeks within the postulated window of male programming was associated with a HR of 1.32 (95% CI 1.04 - 1.69) for cryptorchidism. Exposure to ibuprofen and acetylsalicylic acid was not associated with cryptorchidism.

Conclusions: Data indicate that acetaminophen exposure during the postulated time window where testicular descent is initiated may increase the risk of cryptorchidism. Only limited animal and human evidence supports this hypothesis and replication in other cohorts is needed.

P-048 Sperm DNA integrity after conventional freezing vs vitrification with and without cryoprotectors in oligoasthenospermic patients

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Introduction: Sperm cryopreservation, as part of an assisted reproduction program, is widely utilized in particular in the case of preservation of male fertility before radiotherapy and/or chemotherapy which may lead to testicular failure or ejaculatory dysfunction. Thus, it is possible to offer couples the option of having children in the future. However, due to the damage induced by freezing, the motility of cryopreserved spermatozoa after thawing is statistically reduced.

To date, the problems of cryoprotectant toxicity due to osmotic stress during the addition and removal of cryoprotectants (CPAs) and possible negative effects on the sperm’s structures are unresolved. Our interest has been to verify the effect of high cooling rate of vitrification and the toxic effect of higher concentration of CPAs. In this study we investigated sperm quality in terms of sperm motility and DNA integrity, comparing conventional freezing protocol with vitrification protocols with and without CPAs.

Material and Methods: 18 ejaculates from patients undergoing ICSI, containing at least 10 millions spermatozoa/ml and showing at least 20% total sperm motility were divided into two groups: A and B. Group A underwent conventional freezing protocol with cryoprotectant addition (CPA). Group B underwent vitrification protocol with cryoprotectant addition (CPA) and cryoprotectant removal (CR). Data were analyzed by t-test. The results are presented as means ± SEM.

Results: The concentration of CPAs. In this study we investigated sperm quality in terms of sperm motility and DNA integrity, comparing conventional freezing protocol with vitrification protocols with and without CPAs. Data indicate that acetaminophen exposure during the postulated time window where testicular descent is initiated may increase the risk of cryptorchidism. Only limited animal and human evidence supports this hypothesis and replication in other cohorts is needed.

Conclusion: This work has been supported by a grant from the Spanish Ministry of Education and Science (BFU2006-07779) and IVI Foundation S.L. (Contrato Instituto Sevillano de Infertilidad-CSIC cod.20071101).
motility were obtained by masturbation, after a minimum of 72 h of sexual abstinence.

Informed consent was obtained from each patient. Semen analysis was performed according to the guidelines published by the World Health Organization (1999). Each ejaculate was prepared by swim-up and divided into three aliquots for: traditional freezing protocol with the standard used cryoprotectant Test yolk buffer (TYB), vitrification with (CPAs+) or without (CPAs-) cryoprotectants. Two steps CPAs vitrification protocol was used: sperm suspension was added to a drop of 200 ml PROH 7% + EG 7% for 5a then 1'n in PROH 15% + EG 15% mixing the drops. The suspension was aspirated in a palette for semen cryopreservation and plunged directly in liquid nitrogen. For CPAs-vitrification protocol, 250 ml of sperm suspension was aspirated in a palette for semen cryopreservation and plunged directly in liquid nitrogen.

Warming was performed for all the three protocol thawing the thawed sperm in a 37°C heparin buffered medium. DNA fragmentation was investigated using TUNEL assay. Treatment effects on the parameters assessed were evaluated by T Student test and Chi Square test as appropriate.

The level of statistical significance was set at p < 0.05.

Results: We found a significative sperm motility reduction comparing fresh samples with TYB (47% vs 24%, p = 0.01). Absence of sperm motility was found in CPAs, and lower motility rate was found in CPAs + if compared with TYB (4.6% vs 24%, p < 0.01). The average of DNA fragmentation rate (DFR) in the 18 fresh samples was 21.6%. Interestingly, in fresh samples, DFR was higher in morphological abnormal sperm than in normal ones (57.8% vs 42%, p < 0.01). Higher significant DFR was found in CPAs, CPAs + and TYB compared with fresh samples respectively 52.2%, 48.8 %, 46.1 and 21.6). No difference in terms of DFR was found between the three different freezing protocols.

Conclusion: The vitrification of human spermatozoa in presence or in the absence of conventional cryoprotectants is feasible. We found that sperm motility and DNA integrity is affected by freezing procedure. Motility seems to be completely affected in CPAs- vitrification procedure. The DNA integrity of CPAs+/CPAs-vitrified sperm is comparable with that shown by conventional freezing protocol spermatozoa. Sperm can be used in ICSI fertilization procedure. Vitrification can be used as a quick and simple method. The use of vitrification with CPA allow to obtain motile sperms after thawing. It is necessary to confirm the safety of CPAs-CPAs + vitrification protocol, investigating clinical outcomes after ICSI.

P-049 Nano-toxicity of nano-titanium dioxide in male reproductive system

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Introduction: Nano-particles are differing from larger samples of the same material in their chemical or physical properties. Previous studies have shown that inhalation of nano-sized materials may be harmful for respiratory system. However, only limited knowledge of the toxicity of nano-particles in reproductive system has been reported. Previous study demonstrated that the sperm cell viability could be affected by the presence of gold nano-particles and exposure of diesel exhaust particles (DEP) has been shown to induce leydig cell degeneration, increase the number of damaged seminiferous tubules, and reduce daily sperm production. In this study, the toxicity of the photocatalyst, nano-TiO2, particles, in male reproductive system was examined.

Material and Method: Preparation of murine spermatozoa – we collect mature spermatozoa from cauda epididymis of mice, and purify for motile spermatozoa within centrifugation. The sperm cells were incubated in modified BWW medium for further experiment.

Protein tyrosine phosphorylation – purified sperm cells were incubated with nano-TiO2, (0.01–500 μg/mL) in modified BWW medium within BSA/ Ca2+ activation, then extract cell lysate for electrophorosis analysis and western blotting.

Flowcytometry analysis of sperm cell – sperm cell was incubated with nano-TiO2, then stain for 1'. Cell survival assay - Propidium iodide staining (PI), 2'. ROS production assay - DCFH-DA staining. 3'. Mitochondria integrity assay - CMXROS staining.

Analysis of sperm cell motility via CASA – Sperm cells, pre-incubated with nano-TiO2, were detected by CASA.

In vitro fertilization – Oocytes were collect from super-ovulaton female mice, and then incubated with sperm cells pre-incubated with nano-TiO2. The ratio of two cell was further measured.

Results: The results showed that the level of protein tyrosine phosphorylation of spermatozoa was significantly decreased in mice sperm treated with sub-lethal dosage of nano-TiO2. Protein tyrosine phosphorylation has been well known to be a key signaling which regulate the activities of spermatozoa, such as capacitation, acrosome reaction, and motility. We suggested that the reduction of protein tyrosine phosphorylation may affect the sperm activity. CASA analysis also showed that nano-TiO2 might inhibit sperm motility. In vitro fertilization data showed that the fertilization rate was dose-dependently decreased as the sperm exposure to nano-TiO2. In animal model, after two weeks of nano-TiO2 treatment (directly inject single dose of nano-particles into male mouse testis), we validate the 2-cell embryo ratio via mating to female mice. We found that there is no 2-cell embryo could be found in nano-TiO2 treated group (unfertilized oocytes number = 51, 2-cell embryo = 0), as comparing to the control group (unfertilized oocytes number = 23, 2-cell embryo = 42). We also found certain pathological phenomenon in tests injected with nano-TiO2, as comparing to vehicle control, the nano-TiO2-treated tests showed the accumulation of nano-TiO2 around the seminiferous tubules, and the spermatogenesis showed disruption under exposure to nano-TiO2.

Conclusion: Our findings indicated that nano-TiO2 could affect the male fertility by reducing the activities of sperm. These results also support the petition to pay more attention on nano-TiO2 before introducing into the market.

P-050 Sperm DNA fragmentation is not associated with assisted reproductive outcome

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Introduction: Some contradictory results has been reported about the relationship between sperm DNA fragmentation and IVF outcome. In fact, the routine use of these tests in male infertility evaluation is a matter to discussion. The objective of this work was to ascertain if there is a real effect of sperm DNA fragmentation detected by the terminal deoxynucleobuctide transferase-mediated dUTP nick-end labelling (TUNEL) assay on assisted reproductive outcome in our fertility clinic.

Material and Methods: From April 2008 to july 2009, 181 couples were included in a prospective double-blinded study. Sixty eight couples underwent ICSI due to severe male factor and 113 couples underwent a conventional IVF within our oocyte donation program due to an ovarian failure related to advanced maternal age. At the moment of IVF and ICSI procedures, an aliquot of each capacitated seminal sample (30 μL) was dropped onto slides and air-dried in the IVF laboratory. A code was assigned to each sample, and the slides were given to Instituto Bernabeu Biotech in order to perform the TUNEL assay, using the in situ Cell Death Detection kit with fluorescein isothiocyanate (FITC)-labelled dUTP (Roche). A minimum of 500 spermatozoa were evaluated for each sample. The percentage of TUNEL-positive spermatozoa was referred to as DNA fragmentation index (DFI). Two embryologists (JT and ARA) correlated the code of each slide with the assisted reproductive techniques (ART) information (sperm parameters, fertilization, embryo characteristics, implantation, clinical pregnancy and first trimester miscarriage). They were blinded to the TUNEL assay results. Two molecular biologists (BL and RM) correlated the slide codes with the DFI results and were blinded to ART information. All the data was sent separately to the coordinator of the study (JLL). Statistical analysis comparing ART information with DFI was performed using SPSS 15.0.

Results: In both groups of patients, semen quality (concentration, % forward motility, and morphology), good quality embryos (A plus B), number of embryos transferred, rate of blastocyst formation on day 5 and implantation rate were not affected by DFI. There was no relationship between clinical pregnancy (yes/no) (P = 0.36 and P = 0.99) or first trimester miscarriage (yes/no) (P = 0.40 and P = 0.38) and DFI levels, in the ICSI and IVF recipient groups, re-
spectively. However, while fertilization rate was not affected by DFI values in ICSI patients, a negative correlation was observed in the recipient group \( (R = -0.26, P = 0.01) \).

**Conclusions:** Pregnancy and miscarriage after ART is not affected by sperm DNA fragmentation, measured by TUNEL, independently of semen quality and oocyte origin. The difference in fertilization rate between the two groups could be due to the selection process of spermatozoa before microinjection. According our dates, there is no need to request this test to evaluate the male factor prior to an ART. Notwithstanding, further research is necessary to diagnose sperm at a nuclear level in order to improve the ART outcome.

P-051 **P1/P2 ratio correlates with dynamics of sperm DNA fragmentation**

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**Introduction:** The protamines are the most abundant nuclear proteins present in the mammalian sperm nucleus. Their principal functions are to preserve DNA integrity, to provide a compact hydrodynamic shape and to contribute to the imprinting epigenetics of the paternal genome. In normal conditions, human sperm expresses two different protamines, protamine 1 (P1) and protamine 2 (P2), in almost equal amounts. An altered protamine 1 to 2 ratio (P1/P2) has been described previously in an infertile male and is also related to decreased pregnancy outcome in assisted reproduction techniques (ART). This relationship is not fully understood, but recent papers have shown that a correlation between an altered P1/P2 ratio and sperm DNA fragmentation (SDF) exists. Sperm DNA integrity is a highly limiting factor for the correct transmission of paternal genetic information. This could disturb both fertilization and embryo development processes. Recently, some studies have shown that the SDF can deteriorate rapidly if the semen sample is manipulated (chilled or frozen-thawed) prior to ART. Since it has been demonstrated that different sperm samples from men can exhibit different sperm DNA fragmentation dynamics, the study of dynamics of SDF (VsDF) could give us more accurate information about nuclear sperm vulnerability and susceptibility to DNA damage.

**Material and Methods:** Semen samples from 32 human males (6 control donors of proven fertility, 7 patients who are carriers of structural chromosome reorganization, 9 patients with clinical varicocele and 10 with subclinical varicocele) were analyzed to establish the P1/P2 ratio using acid-urea polyacrylamide gels. In addition, to determine sperm DNA fragmentation, semen samples were incubated 0h, 1h, 4h, 8h and 24h at 37ºC. VsDF was calculated between two different periods of incubation (8–10) using the Sperm Chromatin Dispersion test (SCD). Data analysis was performed using the SPSS 15.0 program. Values were compared by the Kruskal-Wallis test. Correlations were studied using the Pearson test. The level of significance was established at 95% of the confidence interval (IC) to be considered as statistically significant.

**Results:** Statistical differences between patients and the control group according to the P1/P2 ratio \( (p = 0.023) \) and VsDF \( (p = 0.033) \) values were found. Moreover, these two parameters showed a good correlation \( (r = 0.525; p = 0.002) \), and this correlation was better than the correlation obtained for the P1/P2 ratio and basal SDF \( (r = 0.424; p = 0.015) \).

**Conclusions:** The P1/P2 ratio and the VsDF are altered in patients as compared to donors. The P1/P2 ratio correlates with sperm DNA fragmentation, which is consistent with previously published data, but VsDF could be more representative of the abnormal spermiogenesis process than basal SDF. The implication of all of these data could be useful in clinical practice.

**Acknowledgments**

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P-052 **Preimplantation genetic diagnosis for neurodegenerative disorders**

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**Introduction:** If preimplantation genetic diagnosis (PGD) was initially proposed to couples at risk of having a child affected by a severe genetic disorder, late onset diseases such as Huntington’s disease and other neurodegenerative disorders have become a new class of indications. Because of the adult onset of these diseases, prenatal diagnosis (PND) raises numerous ethical issues on the acceptability to terminate an affected pregnancy (TOP). Even if subject to controversy, PGD seems to be a more acceptable option since it does not lead to TOP but consists of selecting embryos free for the mutation. Considering that, in most cases, neurodegenerative diseases are transmitted as dominant trait, PGD is also better accepted by the couple. For at-risk persons who do not want to undergo presymptomatic testing (PT), an exclusion test can be proposed. With such a test, only foetuses or embryos that inherit an allele from the unaffected grandparent are considered as unaffected. Initially the French law specified that the mutation had to be characterised in the parents requesting PGD. The 2004, revised version of the French law authorised PGD if the mutation is characterised in a direct ascendant in case of severe late onset diseases, allowing exclusion testing. We present our experience on 10 years PGD for neurodegenerative diseases in Strasbourg PGD centre.

**Material and Methods:** Between 2000 and 2009, 141 couples were referred for PGD for neurodegenerative diseases: 131 for Huntington disease (HD, 14% of all referrals); half of them asking for exclusion PGD, 7 for spinocerebellar ataxias type 2, 3, 7 or 17, and 2 for pathologies due to prion protein (PRNP) mutations: Gerstmann-Straussler disease (GSD) and fatal familial insomnia (FFI). PGD was accepted for 131 couples. We set up multiplex PCR protocols based either on mutation detection combined with linkage (HD, SCA 2 and 3 and GSD) or on exclusion testing (FFI, HD). After oocyte fecundation by ICSI, embryos were biopsied at day 3 and one or two blastomeres were removed for analysis. Unaffected embryos were transferred on day 4.

**Results:** Between May 2001 and December 2009, 143 cycles were initiated for 63 couples: 137 for HD (72 status known, 65 exclusion), 5 for SCA2 and 1 for GSD. Mean women age at PGD was 31.1y ± 3.5y. Five cycles were performed after successful PGD. Oocyte retrieval (OR) was possible in 119 cycles and 1511 oocytes were collected (mean 12.7 per OR), 599 embryos were biopsied during 119 biopsies and 133 were transferred in 83 transfers (ET) with a mean number of 1.6 embryos per transfer. A positive HCG was noticed in 32 cycles in 32 cycles for 24 couples and 23 clinical pregnancies were observed for 19 couples (21 for HD and 2 for SCA2) Three pregnancies are ongoing. Twenty-six children were born during 20 deliveries: 15 singletons and 5 twins.

Global clinical pregnancy rate is 28% per transfer and 31% per couple with at least one cycle. In case of transfer, each couple has a probability of 56% to initiate a pregnancy and of 44% to have a clinical pregnancy. Clinical pregnancy rates are particularly high in case of exclusion testing for HD: 33% per transfer, 35% per couple with at least one cycle, and 50% per couple with a transfer, respectively.

**Conclusion:** Our experience shows that PGD is a good option for couples at risk to transmit a neurodegenerative disorder to their offspring. This is particularly true when the person at risk does not want to perform premyptomatic testing. In that case, exclusion PGD offers the possibility to transfer unaffected embryos without any information about the status of the person at risk.

P-053 **Human sperm DNA integrity and its relation with anejudropy and semen parameters**

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**Introduction:** Sperm analysis maintains a major role in evaluating male fertility. It is possible to use other techniques such as detecting DNA damage and...
chromosomal numerical anomalies but these are not used routinely. The objective of this study was to compare the chromatin integrity detected by the sperm chromatin structure assay (SCSA) and the sperm chromatin dispersion test (SCD) to evaluate which would be a reliable method to examine DNA sperm fragmentation. Analysis of the chromosomal status of the samples was also examined. It was investigated if there was any relationship between sperm parameters, aneuploidy and DNA fragmentation.

**Material and Methods:** Sperm samples from 109 men were analysed. DNA fragmentation was tested for the 109 samples using a flowcytometry based technique (SCSA) and microscopic based techniques (SCD). Fluorescence in situ hybridization was done for 35 samples to detect aneuploidy (chromosomes X, Y and 18).

**Results:** Using SCSA, the level of DNA fragmentation ranged from 0.5% to 43% (average 11%) and using the SCD it ranged from 0.2% to 57% (average 13%). Using the Pearson correlation in SPSS software there was no significant correlation between the SCSA and SCD methods (P = 0.069). There was no correlation with the semen parameter and DNA fragmentation (P = 0.05) except for the sperm morphology and DNA fragmentation using SCSA (P = 0.031). Sperm aneuploidy ranged from 3 % to 23 % (average 8.6%). Sperm aneuploidy showed no significant correlation with semen parameters and DNA fragmentation (P > 0.05). However, there was a strong relationship with DNA fragmentation detected by SCD (P = 0.008).

**Conclusions:** Although the SCSA and SCD methods measure potential DNA damage and the susceptibility to denaturation, the two methods are distinct and independent. The SCSA assay indicated that DNA fragmentation was correlated with a higher level of abnormal sperm morphology. There was a strong relationship between DNA fragmentation detected by the SCD test with aneuploidy. In conclusion, before such tests are used routinely in the infertility clinic, they should be validated and the clinical significance determined.

**P-054 Seminal plasma protects spermatozoa and pathogenic yeasts from phagocytosis by dendritic cells**

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**Introduction:** Dendritic cells (DCs) are major antigen-presenting cells (APCs) of the immune system and are involved in the coordination of the primary immune response to microbial signals. Their immunomodulatory potential enables them to fulfill regulatory functions in the uterine environment during the process of fertilization and implantation. Seminal plasma functions as a sperm transport medium, but likely also acts as a vehicle for microbial pathogens. Therefore, we investigated not only the influence of seminal plasma on DC-sperm-interaction, but raised for the first time the question, whether seminal plasma effects the interaction of DCs with the human opportunistic fungal pathogen Candida albicans.

**Material and Methods:** DCs were obtained from human monocytes and incubated with labeled sperm cells, C. albicans cells or glass beads in the absence or presence of human seminal plasma. The interaction between DCs and sperm cells, C. albicans or glass beads was analyzed by flow cytometry or fluorescence microscopy. To further characterize the putative binding partners in this interaction, inhibition assays with different components of seminal plasma and antibodies against DC receptors were carried out. Phenotypic changes of DCs in the presence of seminal plasma were further investigated by monitoring the expression of DC characteristic surface markers or by phallolidin-staining of the cytoskeleton.

**Results:** Flow cytometric analysis demonstrated that the interaction between DCs and spermatozoa was significantly reduced in the presence of low concentrations of seminal plasma. To characterize the nature of this inhibitory activity, individual components of seminal plasma are currently tested for a potential inhibitory effect. Further binding studies demonstrated that the DC-SIGN receptor on DCs contributes the sperm-DC interaction. Light microscopy analysis indicated, that seminal plasma also inhibited the association of DCs with C. albicans cells, but not the association with glass beads.

**Conclusions:** In this work we revealed that seminal plasma inhibits the phagocytosis of spermatozoa. This finding supports the assumption that seminal plasma may play a role in human fertilisation and implantation events. In addition, our findings show, that seminal plasma components not only protect spermatozoa from DC action, but they also suggest a putative mechanism of immune evasion, exploited by invading microbial pathogens.

**P-055 Analysis of a catalytic site mutation using mouse PLC zeta-luciferase**

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**Introduction:** Phospholipase C zeta (PLCζ) is a sperm-specific protein that causes Ca2+ oscillations and oocyte activation in all mammalian species. PLCζ liberates IP3, from PIP2, to trigger opening of IP3 gated endoplasmic reticulum Ca2+ channels resulting in Ca2+ release that initiates early embryo development. Recently, a case of male infertility was identified following ICSI treatment and found to be associated with a single point mutation in the PLCζ catalytic Y domain; the substitution of histidine-398 (H398) residue by a proline (H398P) resulted in loss of the Ca2+ oscillation-inducing activity of human PLCζ. This histidine residue is conserved in all mammalian PLCζs, as well as in PLCβ1, a closely related isoform. Here, we have introduced mutations into the equivalent histidine residue of mouse PLCζ and studied the effect upon in vitro enzymatic activity of PLCζ and upon Ca2+ releasing activity in intact mouse oocytes. Luciferase-tagged versions of wild-type mouse PLCζ, PLCζH435P and PLCζH542P mutants were employed to monitor the expression levels of these proteins in mouse oocytes.

**Material and Method:** Human PLCζ His-398 corresponds to His-435 in mouse PLCζ. The previously used pCR3-mluc-PLCζ-luciferase construct was subjected to site-directed mutagenesis (QuikChange, Stratagene) to generate the luciferase-tagged PLCζH435P mutant. We also made a PLCζH542P mutant, to compare how the alternative mutation to alanine would alter the properties of wild-type PLCζ. The cDNA corresponding to wild-type and mutant forms of PLCζ were microinjected into mouse oocytes together with a Ca2+-sensitive fluorescent dye. Luminescence and fluorescence from these mouse oocytes was measured using a photon imaging camera to simultaneously monitor luciferase expression and Ca2+ oscillations, respectively. Recombinant proteins corresponding to the wild-type and PLCζH435P mutant, as well as the wild-type rat PLCζ1 and PLCζ1H435P mutant were expressed in bacteria and assayed for enzymatic activity using a [1H]IP3, hydrolysis assay.

**Results:** The micro injection of cDNA encoding the mPLCζH435P mutant failed to cause any Ca2+ oscillations in mouse oocytes (n = 22), even with a relatively high protein expression level (~230 fg/oocyte). In contrast, wild-type PLCζ cDNA-injected oocytes showed prominent Ca2+ oscillations (n = 17), with the 1st Ca2+ spike occurring at a protein expression level corresponding to 35 fg/oocyte. Likewise, the PLCζH435mutant caused Ca2+ oscillations in all injected oocytes (n = 17), exhibiting a similar potency to wild-type PLCζ, with the onset of Ca2+ spikes detected after expression of 41 fg protein/oocyte. Measurements of the [H]IP3, hydrolytic activity or recombinant protein corresponding to PLCζH435P mutant, showed that it was completely inactive compared to wild-type PLCζ. Similarly, the PLCζ1H435P mutant retained < 20% of the wild-type PLCζ1 activity. Although the PLCζH435P mutant was completely ineffective in causing Ca2+ oscillations, the prior expression of > 10-fold levels of PLCζH435P protein in oocytes did not block the ability of wild-type PLCζ to cause a normal pattern of Ca2+ oscillations.

**Conclusions:** Our findings suggest that the mutation of this conserved PLCζ histidine to a proline, in the Y catalytic domain of mouse PLCζ, dramatically affects the enzymatic activity of the protein, and thus its ability to trigger Ca2+ oscillations in mouse oocytes. In contrast, mutation of this histidine to alanine did not produce a significant decrease in the Ca2+ oscillations-inducing activity of PLCζ. Further, when we investigated the inhibitory effect of PLCζH435P mutant on wild-type PLCζ it was found that PLCζH435P mutant is unlikely to act in a dominant-negative manner.

**P-056 Paraoxonase gene polymorphisms and semen quality**

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**Introduction:** Paraoxonase (PON) is a high-density lipoprotein-associated enzyme that prevents low-density lipoprotein oxidation. PON proteins, localised in the seminiferous tubules and in spermatozoa, have been implicated in the
pathogenesis of male infertility. In the present study, we sought to explore the contribution of the paraoxonase (PON) gene variants to sperm concentration and motility.

**Material and Methods:** One hundred forty five men were examined during IVF treatment, of those 85 men were normozoospermic while 60 were oligozoospermic. DNA was extracted from spermatozoa and the PON1(M/L) 55, PON1(Q/R) 192 and PON2(S/C) 311 polymorphisms were analyzed.

**Results:** The PCR analysis revealed significant differences in genotype and allele distribution of the PON polymorphisms between normozoospermic and oligozoospermic men. Men with oligozoospermia presented less frequently PON1 55S, PON1 192Q and PON2 311S genotypes than normozoospermic men (p = 0.04, p = 0.038 and p = 0.01, respectively). The presence of the PON1 55L, PON1 192R and PON2 311C alleles was significantly increased in oligozoospermic men (p = 0.025, p = 0.018 and p = 0.04, respectively). Furthermore, the PON2 311S allele was associated with enhanced sperm concentration in the total study population (p = 0.008). No significant associations were found between these polymorphisms and sperm motility.

**Conclusions:** The PON1 55L, PON1 192R and PON2 311C alleles are associated with decreased sperm concentration in our series, supporting the significance of PON genes in semen quality.

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**P-057**  
**Is the male chromosomal risk of robertsonian translocations linked to sperm count and/or translocation type?**

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**Introduction:** Robertsonian translocations (RobT) are the most common balanced chromosomal rearrangement (2%). These structural abnormalities are associated to an increased risk of chromosome malsegregation leading to Down syndrome (46,XX,rob(14;21), +21) or Patau syndrome (46,XX,rob(13;14), +13). Furthermore, RobT can be associated to spermato genesis alteration, and explain the increased incidence of RobT in infertile male population.

The aim of this study was to evaluate the aneuploidy segregation and the interchromosomal effect (ICE) rates according to sperm count, and RobT type.

**Materials and Methods:** 22 patients heterozygous for RobT were included in this study 5 were normospemnic and 17 oligozoospermic. In order to evaluate ICE, we also included in this study patients with normal karyotype, 10 were normospemnic and 10 were oligozoospermic. We used sperm FISH with probes for chromosomes involved in the RobT and 13, 18, 21, X, and Y specific probes for ICE evaluation.

**Results:** Studying RobT carriers, sperm aneuploidy rate was lower (p = 0.0054) for the normospemnic patients (9.4%, n = 4,704) than oligozoospermic ones (18.3% n = 14,686) confirming the link between sperm count and sperm aneuploidy rate. Influence of translocation type could only be studied in the group of oligozoospermic RobT carriers (n = 17), and the aneuploidy rate was lower (16.7%) in frequent RobT [rob(13;14) and rob(14;21)] than in rare RobT (25.3%). Reality of ICE was suggested in RobT carriers with normal sperm count where an increase of chromosomes 13 and 21 aneuploidy rates was observed compared to control patients with normal karyotype. This was not the same figure for chromosomes 18, X and Y. ICE could not be demonstrated in the group of oligozoospermic RobT carriers, where no difference was observed compared to oligozoospermic patients with normal karyotype.

**Conclusions:** Translocation type (frequent or rare) and sperm count influence the RobT malsegregation rate and. ICE seems to be restricted to acrocentric chromosomes (13 and 21) and could only be shown in the group of normospermic patients. To confirm the absence of ICE for non acrocentric chromosomes, this study will be extended to chromosomes 3, 4, 7, 9, 12, 16, 17 and 20 segregation analysis and also to 15 and 22 acrocentric chromosomes.

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**P-058**  
**Examination of oocyte activation by calcium ionophore A23187 or strontium chloride in patients with low fertilization rates and follow-up of babies**

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**Introduction:** We have experienced successful pregnancies and births with calcium ionophore (A23187) and strontium chloride (SrCl2) oocyte activation using sperm with repeatedly failed fertilization following intracytoplasmic sperm injection (ICSI). Therefore, in this study, we compared clinical results and physical development of babies born following two different oocyte activation methods.

**Material and Methods:** Subjects of 51 couples were selected from patients who had unsuccessful ICSI treatments between April 2004 and November 2009 at our clinic. The subjects were divided into two groups (Study 1 and 2).

**Study 1**

26 couples who had unsuccessful ICSI treatments sequentially received A23187 after ICSI. 30 minutes after ICSI, the oocytes were placed in 10 mM of SrCl2 medium for 5 minutes, and rinsed, and then cultured. We compared clinical results of these couples without and with oocyte activation.

**Study 2**

25 couples who had unsuccessful previous ICSI treatments sequentially received SrCl2 after ICSI. 30 minutes after ICSI, oocytes were placed in 10 mM of SrCl2 for 10 minutes, and rinsed, and then cultured. We compared clinical results of these couples without and with oocyte activation.

We compared the development and health of 11 babies delivered from 8 couples after oocyte activation. At the time of the study, the babies were between 5 months old and 4 years and 9 months old.

Prior to the child development assessment, we obtained informed consent from the 8 couples and sent written questionnaires to assess the child’s physical and mental development from birth to 3 years according to the Maternal and Children’s Health Handbook issued through local governments by the Ministry of Health and Welfare in Japan. The questionnaires targeted babies’ characteristics at birth, 3, 6, 9, and 12 months, and 1.5, 2, and 3 years of age.

**Results:** Comparing the results without and with A23187 the results were as follows: two pronucleus (2PN) zygote rates, 23.7% (32/135) vs. 53.3% (73/132); P < 0.01; top embryo rates, 27.0% (10/37) vs. 36.4% (20/55); not significant (NS); blastocyst rates, 29.1% (7/24) vs. 31.3% (10/32); NS; expanded blastocyst rates, 16.6% (4/24) vs. 15.6% (5/32); NS; pregnancy rates, 0% (0/8) vs. 20.0% (6/30); NS; and miscarriage rates, 0% (0/0) vs. 6.6% (1/16); NS.

Comparing the results without and with SrCl2, the results were as follows: 2PN zygote rates, 27.2% (37/136) vs. 53.3% (73/132); P < 0.01; top embryo rates, 12.2% (5/41) vs. 26.1% (30/115); NS; blastocyst rates, 4.3% (1/23) vs. 43.5% (43/95); P < 0.01; expanded blastocyst rates, 0% (0/23) vs. 18.9% (18/95); P < 0.05; pregnancy rates, 0% (0/11) vs. 25.6% (10/39); NS; and miscarriage rates, 0% (0/0) vs. 30.0% (3/10); NS.

Comparing the results with A23187 and with SrCl2 there were no significant differences overall.

3 babies from 3 couples were born after A23187 oocyte activation, and 8 babies from 5 patients were born after SrCl2 oocyte activation.

Comparing the results for babies with A23187 and SrCl2, the results were as follows: the length of the gestation periods, 38.7 ± 2.3/38.5 ± 1.3, average birth weight, 3.0 ± 0.3/2.7 ± 0.5, average birth height, 48.1 ± 2.2/47.8 ± 2.5, girth of chest, 30.7 ± 2.5/30.4 ± 2.0, and girth of head, 33.7 ± 0.8/32.5 ± 2.5.

**Conclusions:** Artificial oocyte activation using A23187 or SrCl2 is beneficial in patients with low or no fertility. This study showed that the method of oocyte activation does not adversely affect the growth or health of babies. Further studies are needed to confirm the safety of oocyte activation. As with all assisted reproductive technology treatment, we must inform patients of the risks and benefits of undergoing fertility treatment and possible implications for the future health of their children.

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**P-059**  
**The role of adrenomedullin in regulating human sperm motility**

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**Introduction:** Adrenomedullin (ADM) is a highly conserved member of the calcitonin/calcitonin-gene-related peptide (CRGP)/amylin peptide family. In reproduction, ADM is known to regulate uterine contraction, fetal development.
Materials and Methods: Spermatozoa were obtained from men attending the infertility clinic. A routine semen analysis was performed according to World Health Organization (WHO, 1999). Samples with normal semen parameters were processed by Percoll density gradient centrifugation. The high and low motility fractions were obtained from the 90 and 45% Percoll layers respectively. The binding of Alexa Fluor-555-conjugated ADM to spermatozoon was visualized by cytochemical staining. Localization of ADM receptor components in spermatozoa was demonstrated by immunostaining and western blotting. Hobson Sperm Tracker System was used to study the sperm motility parameters after ADM treatment. Since meta-analysis had shown that the spermatozoa zona pellucida (ZP) binding had a high predictive power for fertilization outcome, therefore, ZP binding capacity will also be studied by hemizona binding assay.

Results: ADM significantly (P < 0.05) enhanced the flagellar beating and ZP-binding capacity of Percoll-processed spermatozoa. At the concentration found in the seminal plasma, it increased the average path velocity, mean straight line velocity, head beat cross frequency, progressive motility and hemizona binding index of human spermatozoa when compared to the control. This action of ADM was completely blocked by the administration of 10× human ADM-γ2 (antagonist of ADM receptors), but not by CGRPγ1 (antagonist of CGRP receptors), suggesting that the ADM receptor plays a predominant role in mediating the observed effects.

Alexa fluor-555-labeled ADM bound to 91.2 ± 2.1% of the Percoll-processed spermatozoa. Strong ADM staining was observed over the neck and the midpiece of spermatozoa. Western blotting and immunohistochemical staining demonstrated for the first time the presence of CRLR and RAMP1-3 on both the neck and the midpiece in human spermatozoa.

Compared with the high-motility fraction, the low-motility fraction after Percoll processing had significantly lower sperm motility and normal form. They also had significantly (P < 0.05) lower level of ADM binding and CRLR, RAMP1 and RAMP3 expressions. Significant (P < 0.05) decrease in ADM binding and CRLR and RAMP3 expressions were also observed in spermatozoa from semen sample with ≤ 30% progressive motile spermatozoa (WHO, grade A + grade B) when compared to those with ≥ 50% progressive motile spermatozoa.

Conclusion: Sperm motility is one of the most important predictors of fertility. It has also been found to be highly predictive of intrauterine insemination success and in vitro/in vivo fertilization rates. The present results suggest that ADM is a factor in the male sex-accessory gland stimulating the progressive motility of human spermatozoa via its classical receptor, CRLR/RAMP. The sperm-ZP binding capacity, an indication of successful fertilization, is also increased by ADM treatment. A significantly negative correlation between ADM binding and sperm motility was consistent with the results. The present investigation provides a physiological basis on possible use of ADM for enhancing sperm motility and the fertilization success. In addition, ADM receptor abundance could be a possible indicator of infertility.

P-061 Anti-müllerian hormone (AMH) serum levels as an independent predictive marker for ovarian reserve and response - considering the benefits for ART

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Introduction: It has been reported that AMH levels are good at predicting ovarian response to IVF ovarian stimulation and as a marker for ovarian reserve. Since AMH are produced at antral follicules (AF) and those levels are stable regardless of the menstrual cycle, it has been suggested to be a valid marker for ovarian reserve, instead of FSH levels and other factors as well as AF. In this study, we investigated the clinical value of AMH as a reference of ovarian reserve and response to control ovarian hysperstimulation (COH), and also AMH levels of patients (pts) group were compared with those of spontaneously conceived women as a control.

Materials and Methods: AMH was measured on 143 informed and consented pts (Mean age: 37.2 ± 4.1, BMI: 21.3 ± 2.8), who came to our clinic between September 2008 and June 2009 for IVF/ICSI, regardless of the menstrual cycle with AMH/MIS EIA Kit (MBL, K.K. Nagoya, Japan) in order to analyze whether there are correlations between the AMH levels and other ovarian parameters, such as age, FSH, AF on the day of menstrual cycle, follicle count and number of collected oocytes after COH. Pts for IVF/ICSI were treated with GnRH agonist and FSH/CGM using either a long or a short treatment protocol, and GnRH antagonist protocol was applied for previous poor respond pts. Single embryo/blastocyst transfer was performed on day 3 or 5 generally (mean no. of ET: 1.1). Pregnancy was defined as the presence of gestational sac(s)/GS. As a control group, the AMH levels of 121 pregnant women (Mean age: 30.9 ± 4.1), who conceived naturally without any experience of infertility treatment, were measured within 20 weeks of gestation.

Results: AMH levels varied between < 3.0 to 62.2(pM/ml) in study group and between 3.4pM to 154.3pM in the control group. There was no correlation in regards to AMH and age both in the study group (r=0.13) and in the control group (r=0.037). Also, no correlation was observed between the AMH of pregnant groups and those of non pregnant groups. However, there was a relative correlation in regards to number of follicle count (r=0.45) and AF (r=0.39). When the study group were subdivided into three groups depending on AMH levels (under 6pM: 30 pts, between 6 and 15pM : 62 pts, over 15pM : 51 pts), significant differences (p < 0.05) were observed between age (39.6 ± 6.1, 37.7 ± 7.7, 35.2 ± 7.9) FSH(12.1 ± 24.8, 5.8 ± 5.2, 4.5 ± 5.2), AF count (3.5 ± 4.6, 5.8 ± 4.7, 8.7 ± 10.0), follicle count (3.7 ± 6.1, 8.0 ± 6.9, 14.4 ± 14.3), number of oocytes collected (4.6 ± 4.9, 8.1 ± 8.9, 13.2 ± 11.3) and rates of pregnancy(10%, 30.65%, 45.1%) respectively among three groups.
P-062 Frequency of LH variant and LQ insert in LH receptor gene among Egyptian males and their association with idiopathic dysfunctional azoospermia

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Introduction: To assess the frequency of LH variant and LQ insert in LH receptor gene among Egyptian males in health control and in cases with idiopathic dysfunctional azoospermia.

Material and Methods: One hundred and fifteen individuals were included in this study divided between 58 healthy fertile control males in their reproductive age and 57 case with idiopathic dysfunctional azoospermia confirmed by two spermiograms and pellet at least two months apart with matched age. Exclusion of other causes of male infertility was confirmed.

All cases and controls underwent a molecular study detection of the allelic variant Trp8Arg/Ile15Thr in the LH β-subunit gene by PCR- RFLP with the restriction enzymes Ngo I and detection of the allelic variant “LQ variant” of the LHR by PCR- RFLP with the restriction enzyme PVU I. Luteinizing hormone(LH), follicle stimulating hormone(FSH), and testosterone levels were measured using ADVIA Centaur CP Immunoassay System.

Results: The genetic variation of the luteinizing hormone β-subunit gene was present at frequency 89.4% and 79.3% for the wild type allele and 15.1% and 19% for the heterozygous alleles and 0% and 1.7% for the homozygous allele of LH variants in cases and healthy controls respectively with insignificant difference between both groups.

The “LQ variant” of the LH gene was present at frequency 54.4% and 50% for the wild type allele and 42.1% and 36.5% for the heterozygous alleles and 3.5% and 13.5% for the homozygous alleles of LH variants in cases and healthy controls respectively with insignificant difference between both groups.

There was a significant difference between the levels of LH(p: > 0.001), FSH (p: > 0.001)and testosterone(p: > 0.005) between both groups.

Conclusions: The frequencies of Trp8Arg/Ile15Thr in the LH β-subunit gene and the “LQ variant” of the LH gene in both groups exclude a relationship between these variations and idiopathic dysfunctional azoospermia. Other factors should be evaluated to assess the significant difference in the hormonal levels.

P-063 Is there a correlation between head sperm morphology and DNA abnormalities?

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Introduction: In a previous study*, we showed that low scoring spermatozoa selected under high magnification (X 6100) during ICSI were associated with low fertilization rate.

More precisely when spermatozoon with a score 0 was injected; no embryo development to expanded blastocyst stage and no pregnancies were noticed.

The objective of the present study is to examine the correlation between score 0 spermatozoa and DNA abnormalities in sperm: AneuPloidy, DNA fragmentation and chromatin condensation.

Material and Methods: Fresh semen samples from 96 men referred to our center for ICSI were washed using density gradient centrifugation. The percentage of score 0 spermatozoa in each samples was determined. In the first 26 patients; spermatozooa scoring 0 according Cassuto and Barak classification were selected at high magnification and set on 3 separate slides, next to a control of all washed spermatozooa for each sperm sample.

The 3 slides were then each used for: - AneuPloidy study using fluorescence in situ hybridization (FISH) was performed with X, Y, and 18 centrometric chromosomes probes.
- DNA fragmentation was evaluated using the terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay
- Sperm chromatin condensation was evaluated by Bleu Aniline staining.

In total we analyzed 7800 score 0 spermatozooa and the same number of corresponding controls. Correlations were studied between score 0 spermatozooa and DNA anomalies.

Chromatin condensation was then performed in all 96 patients and correlated to score 0 spermatozooa.

Results: DNA fragmentation rate and aneuploidy were comparable between spermatozooa score 0 and controls. The rate of chromatin condensation was two times higher in score 0 then in controls (19.5% vs. 10.1%, p < 0.0001). Data from the 96 patients showed a positive correlation between chromatin condensation and the percentage of spermatozooa with a score 0 in gradient selected semen (r = + 0.24 p = 0.02).

Conclusions: The importance of sperm DNA integrity in sperm function and success rates in assisted reproductions is already established. In the present study, we discovered that score 0 spermatozooa are correlated to chromatin condensation rather than DNA fragmentation or aneuploidy. We therefore advised a simple and effective method (Cassuto and Barak score) that can be used to select and eliminate spermatozooa just before injection in ICSI with high level of chromatin condensation to improve ICSI outcome.

P-064 The role of leucocyte subpopulations in male sub fertility

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Introduction: Leucocytospermia, a frequent finding in subfertile patients is often associated with poor semen quality. Analysis of the heterogenous aetiology of the leucocytes populations in human semen is useful not only in the diagnosis and therapeutic monitoring of male genital tract infections including sexually transmitted diseases but also for autoimmune diseases. This study aimed to identify the role of leucocytes and predominant leucocyte subpopulations in male sub fertility. The study also aimed to determine the role of natural killer cells in male sub fertility. The study will help to clarify a much debated issue.

Materials and Methods: A prospective cohort study of the leucocyte subpopulations of seminal fluid of different categories of subfertile men was undertaken. Men recruited into the study were divided into 5 groups: Normospermia (n = 14), Asthenospermia (n = 10), Oligospermia (n = 13), Oligoasthenospermia (n = 19) and Obstructive Azoospermia (n = 10), based on their semen analysis.

The remainder of the sample underwent immunocytochemical staining, the monoclonal antibodies used in this study include: CD3, CD4, CD8 (T Cells), CD14 (monocytes/macrophages), CD16 (granulocytes), CD20 (Bcells), CD45 (Pan Leucocytes), CD56 (Natural Killer cells) and CD69 (Activated T and B cells).

The number of positively stained (red-pink) leucocytes for each antibody were counted in ten high power fields (HPF = x 320 magnification) using a light microscope fitted with a graticule. The median leucocyte counts of the subfertile groups were compared to the values in the normospermic group. Analysis of the data was undertaken with SPSS v13.

Results: Significant levels of CD3 helper T lymphocytes (p < 0.001) were present in the oligospermic, asthenospermic, oligoasthenospermic and obstructive azoospermic group compared to the normospermic group. Significant levels of B cells (p < 0.05) were present in the asthenospermic, oligoasthenospermic and obstructive azoospermic group. The natural killer cells (CD56) were significantly raised in the oligoasthenospermic and obstructive azoospermic group (p < 0.05).
Conclusion: This is the first study to have identified significant levels of CD3 helper lymphocytes and B cells in the various subfertile groups. Our findings suggest that leukocytospermia could impair sperm function through enhanced T helper cell modulation. An increase in the B cell population in the groups may result in increased levels of antisperm antibody being secreted which could impair sperm numbers and function which would result in subfertility. The presence of various leukocyte subpopulations in the obstructive azoospermic group suggests that the site of seminal leukocyte production is not necessarily confined to the vas or the epididymis as previously thought. Natural killer cells could be responsible for mediating sperm damage especially in the oligoasthenospermic and obstructive azoospermic group. The presence of increased level of lymphocytes in the reproductive tract could be as a result of an immunological reaction. Although, other authors have suggested that semen parameters including progressive motility rate and sperm concentrations were significantly lower in the leukocytospermic group compared with the control group, this is the first study that defines the type of leukocyte subpopulations in subfertile groups.

References:

P-065 Malonaldehyde (MDA) formation and DNA fragmentation: two independent ROS induced sperm decays

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Introduction: Malondialdehyde (MDA) formation in spermogenesis is a process resulting from membrane lipid peroxidation. Sperm DNA fragmentation is mainly the result of Reactive Oxygen Species (ROS) insults. We have tried to determine if there is any correlation between MDA content, WHO parameters, and DNA fragmentation in patients seeking consulting for hypospermatogenesis.

Material and Methods: The work received approval from the ethical committee of the three private clinics and the two laboratories and involved 163 patients having signed an informed consent. After liquefaction, sperm was analyzed according to WHO guidelines. The remaining sample was aliquoted and frozen for further analysis, which included sperm DNA fragmentation assay with TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) and sperm decondensation assay with aniline blue. Sperm lipid peroxidation level was assessed using measurement of Thiobarbituric Acid Reactive Substances (TBARS). In this study we deliberately did not separate sperm and seminal plasma in order to avoid any oxidative interference that classically occurs during sperm processing (i.e. centrifugation in high oxygen tension and removal of reducing compounds of the seminal plasma). The relationship between MDA and other sperm markers was analysed by the way of correlation coefficients.

Results: The classical correlations between age and sperm DNA fragmentation (p < 0.001), and between sperm concentration and immaturity (decondensation p < 0.04), were found, indicating no bias in our patient sample.

No correlation was found between DNA fragmentation and MDA formation (mean values between 6.5 and 7.5 nmo/l/mL). Thus, our data show no correlation between DNA fragmentation and lipid peroxidation (p = 0.88). This suggests that there are two independent steps: 4-Hydroxy-2-nonenal (HNE) and MDA formation.

Conclusions: This study provides evidence that malondialdehyde (MDA) formation in sperm is not dependent on DNA fragmentation and that these two metabolic events are independent.

P-066 High incidence of protoplasmic abnormalities in human testicular spermatozoa: implications for sperm selection at ICSI

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Introduction: The selection of the fertilizing spermatozoon during ICSI is a crucial step especially in the case of surgically retrieved testicular spermatozoa. In these circumstances, the range of selection is often very limited because of the scarce presence of motile spermatozoa with normal morphology. The aim of this study was to verify whether the analysis by polarizing microscopy of the birefringence characteristics of spermatozoa as a reflection of their protoplasmic structure could add valuable information in relation to sperm morphology assessed in the same samples used for the ICSI procedure. For this reason, only samples with immotile spermatozoa retrieved by the male seminal tract were analyzed.

Materials and Methods: This study included a total of 345 thawed spermatozoa from 7 cryopreserved samples surgically retrieved in azoospermic patients. Cells were analyzed for morphology and birefringence characteristics using an inverted microscope equipped with polarizing lenses and enhanced digital imaging. The patterns of birefringence were evaluated in each sperm cell by capturing the corresponding images in the computer that also permitted an accurate classification of sperm morphology assisted by enhanced magnification. The examination of cells was performed by a 63X objective, while the total magnification using the enhanced digital imaging on the monitor was between 2500X and 5500X. In relation to birefringence, two groups of samples were distinguished: sperm with a total birefringent head due to the presence of an intact acrosome and an organized nucleus, and sperm with a partial birefringent head in which birefringence was localized in the post-acrosomal region. Sperm morphology was assessed according to WHO criteria by evaluating: A) head (shape, dimensions, vacuoles, nucleus, acrosome), B) neck-midpiece, C) tail, D) presence of cytoplasmic-droplets.

Results: In the population of spermatozoa with a normal morphology (33%), 54% showed a total head birefringence, 26% had partial head birefringence, and 20% were no birefringent. This distribution was significantly different in sperm cells with an abnormal morphology (66.9%), in which total birefringence was detected in 21.6% of cells (P < 0.001 vs. sperm with normal morphology), partial birefringence in 19.4% of cells (P < 0.01), and 59% of cells were devoid of birefringence (P < 0.001).

The main morphology alterations were observed in the head (41%). In this subgroup, the proportion of non birefringent spermatozoa (71%) was significantly higher than in morphologically normal spermatozoa (P < 0.001). Similar results were obtained in the subgroup of spermatozoa with neck-midpiece defects (14.7% of abnormalities), tail defects (23.4%) and cytoplasmic-droplets defects (18.1%), in which absence of birefringence was found in 65%, 45% and 33% respectively of the analyzed sperm cells (all these differences were statistically significant at P < 0.05). The proportion of totally birefringent spermatozoa was higher in morphologically normal cells (54%) when compared to all subgroups of abnormal morphology: 11% for head defects (P < 0.01), 12% for neck and midpiece defects (P < 0.001), 45% for tail defects (P = 0.005) and 48% in case of cytoplasmic droplets (P = 0.55). Regarding the frequency of partial birefringence, there were no significant differences between spermatozoa with normal morphology (26%) and those with morphological defects (head 17%, neck-midpiece 23%, tail 9%, and cytoplasmic-droplets 20%).

Conclusions: For immotile testicular spermatozoa, those with normal morphology have the highest chances of possessing normal protoplasmic structure.
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P-067 Screening for spermatogonia stem cells in NOA patients
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Introduction: Azospermia affects about 10% of all infertile men. While obstructed azoospermic men had their spermatogenesis preserved and spermatogenesis can be retrieved either through the epididymis or testes, non-obstructive azoospermic men on the other hand, yield spermatozoa in only 60% of the cases. Here we assessed patient with secretory azoospermia where few scant spermatocytes were identified after testicular biopsy were planned for ICSI but failed to produce spermatozoa. We investigated the feasibility of assessing the residual germ cells in digested cell suspensions and seminiferous tubules fragments.

Materials and Methods: Testicular biopsy specimens were obtained from men who underwent infertility treatment and donated their remaining samples. For ICSI, TESE samples were enzymatically digested and assessed for spermatozoa or other germ cells. When spermatogenesis were absent, smears were made from cell suspensions and leftover intact tubules were fixed, embedded in paraffin, and micromotomed to obtain 3 μm sections. Samples were stained with VASA (primordial germ cell to early spermatid), DAZL (A spermatogonia), vimentin (Sertoli cells), and 3-b-hydroxysteroid dehydrogenase (3-b-HSD; Leydig cells).

Mouse testes were obtained from adults (12 - 18 wks) and processed in the same fashion. The criteria used to identify the spermatogenic cell types within the seminiferous epithelium were those of Russel et al. (1990).

Results: From September 1993 to January 2010, ICSI was performed on 15,277 (89.2%) cycles with ejaculated, 836 (4.9%) with epididymal, and 1,010 (5.9%) with testicular spermatozoa. Of the testicular biopsies, the 870 (86.1%) succeeded as NOA and 140 (13.9%) were OA. Of the NOA schedule for ICSI, 9.4% (n = 95) failed to produce any spermatozoa after several hours of extensive specimen search by several embryologists. In order to assess whether spermatogenesis were absent or were originally absent due to spermatogenic arrest or germ cell aplasia, we proceeded to test for germ cell identification by staining slides smeared with testicular cell suspension. When available, tubules (intact or minced) were also processed.

In order to acquire expertise in germ cell identification, we processed 24 testes from adult mice that had an average size of 8.8x3mm and weighed about 118 ± 8mg. After staining with VASA we were able to identify different stages of the germ cells, from type A adjacent to the basal membrane followed by type B more centripetally towards the lumen. Spermatocytes in different developmental stages matured and migrated in the same direction. Early spermatids were stained with DAZL, a ring of type A spermatogonia was located next to the basement membrane. Sertoli cells appeared as finger-like projections going towards the lumen. Leydig cells were 3-4 cells interspersed in between the external tubular walls.

A total of seven leftover testicular biopsies from NOA men were processed, of which 4 were from cell suspensions and 3 from small tubular fragments ranging in size between 2 and 5mm. In 2 cell suspensions, we were able to count 606 cells, inclusive of 44 (7.3%) spermatids, 128 (21.2%) spermatocytes, and 25 (4.1%) spermatogonias. When intact tubules were evaluated, Sertoli and Leydig cells had the same appearance and disposition as in the mouse—VASA was only able to distinguish a scant number (n = 18) of spermatogonia and of those only 2 cells were positive for DAZL but only in 1 specimen.

Conclusions: The information gained in lower mammals proved critical for the understanding human spermatogenesis. Germ cell identification testing demonstrated that extensive sperm search to be reliable in identifying spermatogonia in testicular biopsy of non-obstructive men. This test in combination with extensive sperm search at time of treatment facilitate differentiation between germ cell aplasia versus spermatogenic arrest.

P-068 Frequent biological hypogonadism of various origins in males affected by testicular cancer
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Introduction: Hypogonadism in males is a complex clinical syndrome defined by low level of testosterone and diminished sperm production. Some authors reported a high incidence of hypogonadism after cancer treatment in the childhood. In testicular cancer, post-treatment hypogonadism has been suggested to be more frequent if present already before cancer treatment.

At our institution a longitudinal prospective study on fertility after testicular cancer is underway with the aim to increases the understanding of sexual behaviour and fertility in men who had survived testicular cancer. The preliminary results presented here concern the hormonal status of the patients.

Material and Methods: Among men who were referred for sperm cryopreservation before treatment of testicular cancer (T0) between January 2007 and December 2008, 53 men aged between 19 and 42 years accepted to participate in the study. Fifty one men had hormone assessment done including FSH, LH, total testosterone (TT), SHBG and free testosterone index (ratio TT/SHBG, FTI); hormone assessment was repeated at one year (T1, n = 20). All tumors were germ-cell. Males were assessed immediately before (n = 4) or after orchiectomy (n = 49). Increased levels of gonadotropins indicated primary hypogonadism, low levels of gonadotropins were indicative of secondary hypogonadism. Men were clinically examined and sperm analysis performed.

Results: At T0, 5 men were affected by primary hypogonadism with low testosterone and 9 (18.4%) by secondary hypogonadism; overall 17 (28.5%) men had biological hypogonadism.

Additionally, 7 men had primary hypogonadism with normal testosterone and 19 men had normal hormone levels. No difference between the four groups was found regarding the male age, previous fertility status, testicular volume and sperm characteristics.

Only 9 men came to a follow-up visit 1 year later. In 6 hormone levels were normal at T0 and remained normal, 3 were normal at T0 but developed primary hypogonadism with low testosterone at T1. In further 11 men hormones were first assessed at T1; 6 had primary hypogonadism of which 3 (27.3%) had low testosterone.

Conclusions: Hypogonadism is frequently observed in patients affected by testicular cancer even before any treatment toxic for germ cells. Clinically, hypogonadism is difficult to diagnose because andrological history (cryptorchidism), general examination and testicular volume are not characteristic. Moreover, the affected men in our study did not differ from hormonally normal men regarding sperm quality. Careful attention must be paid to subjective signs such as asthenia and diagnosis confirmed by laboratory tests. At T0, men are more affected by secondary hypogonadism (role of stressful orchidectomy?), whereas at T1 more cases of primary hypogonadism are observed (role of cancer treatment?).
Introduction: Spermatozoa with large vacuoles detected with Motile Sperm Organelle Morphology Examination (MSOME) may have a negative impact on embryo development. Origin of these vacuoles and structures involved in their genesis are unknown. The aim of our study was to evaluate acrosome and nucleolar alterations on isolated spermatozoa with large vacuoles, compared with spermatozoa from whole sperm.

Material and Methods: Twenty patients with teratozoospermia were included in our study. Spermatozoa from whole sperm and spermatozoa presenting a vacuole occupying more than 13% of the total head area, isolated under high magnification microscope (= 6000), were assessed. Confocal and transmission electron microscope evaluations were carried out on isolated spermatozoa and whole sperm, respectively. Acrosome morphology and DNA fragmentation were analysed using a method combining proacrosin immunolabelling with monoclonal antibody Mab4D4 and TUNEL assay. Chromatin condensation was evaluated with aniline blue staining. Sperm aneuploidy was assessed using fluorescent in situ hybridization (FISH).

Results: Spermatozoa with large vacuoles represented 38% ± 5.10 of the spermatozoa. Vacuoles were mainly located in anterior and median parts of sperm head (45.7% ± 2.90 and 46.1% ± 3.00). A significant increase of abnormal acrosomes was observed in spermatozoa with large vacuoles compared to whole sperm (77.8% ± 2.49 vs 70.6% ± 2.62; p = 0.014). However, electron and confocal microscope observations concluded to an exclusive nuclear localization of these vacuoles. Total DNA fragmentation was significantly higher in whole sperm (p < 0.0001). On the contrary, chromatin condensation was significantly altered in spermatozoa with large vacuoles (p < 0.0001). Aneuploidy and diploidy rates were also increased in spermatozoa with large vacuoles (p < 0.0001).

Conclusion: The association between abnormal acrosome, immature chromatia and intranuclear vacuoles supports the hypothesis of a common origin of these abnormalities occurring during spermiogenesis. However, the increase of aneuploidy in large vacuole spermatozoa also suggests a possible premeiotic or meiotic chromatin disturbance responsible for abnormal sperm chromatin organisation and vacuole formation.

P-072 Semen quality at cryobanking before oncologic treatment
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Introduction: Sperm cryobanking is a standard method of choice to preserve fertility of men undergoing oncologic treatment. Chemotherapy, radiation or their combination results in a significant reduction of sperm quality. However, it is also suggested that the disease itself influences spermatogenesis. Testicular cancer (TC) and Hodgkin disease (HD) patients represent 80% of all cryopreservation in our laboratory and the 5 year survival rates are 95% and 84%, respectively. Thus, they form the largest group of reproductive importance. In this study we investigated (1) the effect of the malignant disease on testicular sperm extraction suffer from severe hypospermatogenesis and from seminal duct obstruction (epididymo-testicular disconnection, segmental atresia of epididymis...), like classically described in cryptorchid boys during orchidopexy.
from 1993 to 2009. All TC patients were evaluated within 1 month after unilateral orchidectomy. Sperm parameters (ejaculate volume, sperm concentration, total sperm count, forward motility) were evaluated according to WHO 1999 guideline.

**Results:** The mean age and sperm concentration was 27 years (range 16–42), 21.69 M/mL (± SD 20.5) in the TC group and 25 years (range 16–34), 31.35 M/mL (± SD 23.6) in the HD group. In the 104 TC patients 48 (46.1%) oligozoospermic and 13 (16.2%) azoospermic individuals were detected. Only 43 (41.3%) of them had more than 40 million total sperm count/ejaculate. The average forward motility was 26.7% (± SD 5.4). Histological data were available for 65 TC patients. The sperm concentration was reduced both in seminoma patients (N = 32, 22.64 M/mL ± SD 15.5) and in non-seminoma (embryonal carcinoma, yolk sac tumors, choriocarcinoma) TC patients (n = 33, 22.3 M/mL ± SD 16.5). Thirteen TC patients and six HD patients were azoospermic. Among the 45 HD patients 14 (31.2%) were oligozoospermic, 6 (13.2%) were azoospermic and only 25 (55.5%) normozoospermic. The average forward motility was 25.9% (± SD 5.56).

**Conclusions:** Spermatogenesis is defected in most testicular cancer patients, and Hodgkin disease also negatively influences the sperm parameters. On average, normal sperm count can be detected only in half of the oncologic patients apply for sperm cryopreservation. The decreased progressive motility suggests that with the reduced sperm concentration the sperm function is also affected, suggesting decreased fertilizing potential. Functional testing, chromatin assays and chromosomal analysis of samples would improve the assessment of the fertility prognosis of these patients before cryopreservation.

**P-073 Sperm apoptotic markers as related to clinical and hormonal characteristics, and fertility potential**

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**Introduction:** The origin and significance of apoptosis markers changes in ejaculated sperm are debated.

**Material and Methods:** Semen and serum samples from men of infertile couples were evaluated. The study involved the determination of classical sperm characteristics in neat semen and sperm marker changes i.e. changes in plasma membrane phospholipid asymmetry, mitochondrial membrane potential (MMP) and DNA integrity by SCSA in semen prepared by density gradient using flow cytometry. Serum FSH was measured by a solid-phase, two-site chemiluminescent immunometric assay. Couples were followed for 36 to 60 months. In the study we enrolled 143 couples and divided them according to the method of conception: in 76 the female partner could conceive naturally or by ART, but two.

**Results:** Male age was significantly correlated to the number of non apoptotic cells (r = -0.244, p = 0.004) and to DNA denaturation (r = 0.206, p = 0.014). Testicular volume was correlated to non apoptotic cells (r = 0.259, p = 0.002), MMP (r = 0.295, p < 0.001) and DNA denaturation (r = -0.168, p < 0.045). FSH was correlated to apoptotic cells (r = 0.227, p = 0.008) and MMP (r = -0.262, p = 0.002). A significant correlation was observed between apoptotic cells, cells with normal MMP, DNA denaturation and classical sperm characteristics. In men whose partners conceived naturally or after IUI sperm DNA denaturation was significantly lower in comparison with those who did not conceive (7.7% ± 2.2% vs. 19.1% ± 2.3%, p = 0.024). Women whose male partner had sperm DNA denaturation > 10% had four times lower chance to conceive naturally. In men undergoing IVF or ICSI, sperm apoptotic markers were not predictive of pregnancy.

**Conclusions:** Changes in plasma membrane phospholipid asymmetry, mitochondrial membrane potential (MMP) and DNA integrity are related to male age and important indicators of spermatogenesis, i.e. to testicular volume and serum FSH. Though apoptotic markers do not predict pregnancy in IVF and ICSI, they predict natural pregnancy. Therefore, sperm apoptotic markers provide a clinical indication for routine use in infertility evaluation.

**P-074 DNA fragmentation and semen alterations in elite triathletes**

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**Introduction:** One of the sports modalities that has gained greater popularity in the last years is triathlon. Its practice demands high physical requirements from its participants, making the athletes undergo high training volumes and competitions of long duration in the modalities of cycling, swimming and running.

Among the different varieties of triathlon, the most exhausting is the Ironman, where athletes compete to swim 3800m, pedal for 180km, and run 42km, this means that high level athletes have to spend between 9 to 10 hours performing physical exercise in a continuous manner. It is easily understood that such competition imposes an elevated stress on the athlete’s organism.

Previous studies have related this sports practice to semen alterations (Vaamonde et al., 2009), especially when training volume is high. The observations lead us to hypothesize that significant alterations in DNA may also be present. Therefore, the objective of the present study was to assess DNA fragmentation and semen parameters in high-level triathletes.

**Material and Methods:** Nine high-level triathletes voluntarily participated from the present study. Semen and training parameters were carefully analyzed. The subjects, aged 27 ± 3 had been practicing triathlon for 5 years in average. They have a mean VO2max above 70 ml/kg/min. In brief, their mean annual training regime is as follows: 416km of swimming, 13000km of cycling, and 2600km of running. The mean values for the last week of training undertaken by the athletes composing the sample were: 4km of swimming, 220 km of cycling, and 14 km of running.

The triathletes were asked to keep the standard days of abstinence for proper semen assessment. Likewise, indications were also given regarding coffee, drugs, and alcohol consumption. In addition, and as a preventive measure, they considerably diminished training volume during the last week, and especially for two days before semen collection in order to avoid an acute effect on semen quality. On the day of semen collection, subjects completed a questionnaire ruling out any possible effect on the HPG axis for reasons other than sports practice.

Sample normality was assessed following standard WHO values for volume, concentration and total number, and velocity; morphology normalcy, on the contrary, was assessed following Kruger’s strict criteria. For DNA fragmentation evaluation, we used the sperm chromatin dispersion (SCD) test.

**Results:** Mean values for volume, concentration, and velocity were among normal ranges. However, both morphology normalcy and DNA fragmentation showed abnormal values (5.3 ± 2.7%, and 20.4 ± 6.1%, respectively). Additionally, we observed an excess of round cells above normal range in all samples but two.

**Conclusions:** The altered value observed in the sperm fragmentation test supports the hypothesis that high loads of endurance training alter semen parameters and may interfere with the athlete’s fertility potential.

**References:**

**P-075 The importance of carefully screening the ejaculate in non-obstructive azoospermic men**

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**Introduction:** At time of initial consultation for infertility, a semen analysis is a relevant component of the male work-up. When no spermatozoa are seen in a counting chamber, often a centrifugation of the specimen is required to confirm
Sperm suspensions from consenting men undergo hormonal (seminiferous tubules. The mixed population of cells obtained cultured on 24 (4, 8, 12 week-old mice - BALB/c) and sexually immature mice (1, 2 week-

Materials and Methods: From January 2002 to December 2009, 9,106 men were screened and 356 were diagnosed with azoospermia, initially by first observing the sample in one counting chamber. After the initial concentration was confirmed to be zero, the sample was centrifuged at 2000-3000g for 10-15min and observed again in triplicate. Men with no spermatozoa were then scheduled for TESE but on the day of surgical retrieval, the ejaculate was again screened first. Spermatozoa found during the extensive sperm search on the day of egg retrieval were used for ICSI.

Results: A total of 145 azoospermic men who underwent 181 ICSI cycles were evaluated. The average age of the men that had any ejaculated spermatozoa was 38.8 ± 7 yrs and their female partners were 35.5 ± 9 yrs. Among the couples that used donor spermatozoa the females had a mean age of 35.3 ± 4 yrs. Twenty-two of the azoospermic men opted to use donor spermatozoa immediately, with the remaining being scheduled for TESE. However, at the time of ICSI, spermatozoa were found in 17 (9.9%) ejaculates and 102 men proceeded with 142 (78.5%) testicular biopsies. Spermatozoa were recovered in 112 (78.9%) cycles, either immediately or after an extended search of the TESE samples. Men that had no spermatozoa in their seminiferous tubules (n = 14) chose to use donor sperm.

The fertilization rate was 51.3% (81/158) using sperm found in the ejaculate and 53.2% (67/1,262) with TESE sperm, and with clinical pregnancy rates of 41.2% (7/17) and 38.4% (48/125), respectively. In patients that utilized donor spermatozoa (n = 36), the fertilization rate was 75.7% (250/332) with a clinical pregnancy of 50.0% (18/36). Pregnancy losses reached 11.8% (2/17) in the ejaculated group, 10.7% (12/112) after using TESE sperm, and 16.7 (6/36) with donor sperm.

Conclusions: Physiological fluctuations in the spermatogenetic process mean that putatively NOA men can still sometimes yield ejaculated spermatozoa. Performing an extensive sperm search can allow these men to achieve successful fertilization and pregnancy. Screening ejaculates prior to a TESE procedure is still advisable in patients with secretory azoospermia.

P-076 Effect of age on the capacity of mouse Sertoli cells to produce GDNF, SCF, LIF, CSF-1 in vitro

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Introduction: Spermatogenesis is regulated by Sertoli cells (SCs) which produce various factors that are required for development of germ cells (GCs). The number of SC determines testicular size, GC numbers per testis and spermatozoa output. Furthermore, SC provides a specialized, protected environment within the seminiferous tubules of the testis for GC development. SC provides factors required for GC metabolism and growth/development such as stem cell factor (SCF), glial cell line derived neurotrophic factor (GDNF), leukemia inhibitory factor (LIF) and colony stimulating factor (CSF-1). Within the seminiferous tubules only SC possess receptors for testosterone and FSH and thus these cells are the major targets of the ultimate hormonal signals that regulate spermatogenesis. The aim of this study is to evaluate the capacity of SC during mouse sexual maturation to produce growth factors such as GDNF, LIF, SCF and CSF-1.

Material and Methods: SC were isolated from testes of sexually mature mice (4, 8, 12 week-old mice - BALB/c) and sexually immature mice (1, 2 week-old mice - BALB/c) by decapsulation and enzymatically dissociation of the seminiferous tubules. The mixed population of cells obtained cultured on 24 wells plate with DMEM and 10% FCS then incubated at 37°C with 5% CO2 for 4 days and then was treated with hypotonic solution for 5 minutes at room temperature to remove residual GCs. The hypotonic solution was removed by gentle aspiration and replaced with fresh medium for 2 more days of incubation at 37°C with 5% CO2. SC were cultured in the absence (-H) or presence of hormones (+ H) [FSH (5 IU/ml) + testosterone (1 nM)] for 8 hours. The media were collected and stored at -70°C until used for GDNF and LIF analysis by ELISA. Also, RNA was extracted from cultured SC to evaluate the expression of the above factors by real time PCR analysis.

Results: Our results show that cultures of SC from sexually immature mice (1 week), in the absence of hormones (-H), expressed and secreted significantly higher levels of GDNF, LIF and SCF as compared to SC from other ages (2-12 week-old). The levels of GDNF, LIF and SCF produced by SC of 2-12 week-old mice, were significantly lower as compared to SC from 1 week-old mice, in both RNA and protein levels. However the levels of CSF-1 produced from SC of 1 week-old mice, in the absence of hormones, were in basal levels. A significant increase in the levels of CSF-1 produced by SC from 2-12 week-old mice was detected, this increase in CSF-1 was age-dependent (from 1 week-12 week). Addition of hormones (+ H) to SC cultures significantly decreased the levels of LIF, SCF and GDNF (non-significant) both in RNA and protein levels. On the other hand, the levels of CSF-1 were significantly decreased only in SC cultures from 12 week-old mice as compared to SC from 1 week-old mice.

Conclusions: The results indicate that the capacity of SC to secrete SCF, GDNF and LIF were decreased but increased for CSF-1 during mouse development. Hormones are one of the factors that involved in this regulatory process. Thus, the present study may suggest that the balance and/or time tuning of the different growth factors production by SC are regulating testicular germ cells growth/development.

P-077 Concurrent assessment of DNA breakage in the sperm nucleus

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Introduction: Sperm DNA integrity and proper chromatin packaging are required for normal functioning of the male genome within the oocyte. The notion that sperm DNA damage may affect embryo development and generate epigenetic disorders has led us to evaluate different assays considered to measure the competence of the sperm nucleus. However, these methods appear often to be discordant, inconsistent and fail to predict outcomes, particularly when few spermatozoa are available.

Therefore, in this study we have assessed and compared the ability of different assays to detect DNA breaks and nicks in the same spermatozoa.

Material and Methods: Sperm suspensions from consenting men undergoing screening for male infertility were subjected to the SCD test and TUNEL assay performed in-house according to manufacturer’s protocol, and were sent outside laboratory for SCSA analysis. In the case of the SCD and TUNEL assays, thresholds of 18% and 15% were adopted respectively, while a DFI of 30% was used for the SCSA.

To simultaneously perform SCD and TUNEL assays on the same spermatozoa, aliquots of the washed specimens were embedded in 1% agarose at 37°C. A total of 20µl of the semen-agarose mix was pipetted onto precoated slides (Halosperm™), coverslipped, and left to solidify at 4°C. After gently removing the coverslip, slides were immediately treated with 0.08N HCl to generate single-stranded DNA breaks. Slides were then exposed to a lysing solution, to remove the nuclear proteins and dehydrated in an ethanol series. Once dried, the slides were incubated with TdT and BrdU/TP (APO-BrdU™ TUNEL Assay Kit) for the detection of DNA breaks. Detection of BrdU incorporation was achieved through anti-BrdU antibody labeled with a fluorescent dye. Propidium iodide was used as counterstain. A minimum of 500 spermatozoa/slide were analyzed through anti-BrdU antibody labeled with a fluorescent dye. Propidium iodide for the detection of DNA breaks and nicks in the same spermatozoon.

Therefore, in this study we have assessed and compared the ability of the different assays to detect DNA breaks and nicks in the same spermatozoa.

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Results: A total of 291 specimens from 241 patients were screened for DNA fragmentation by SCSA, SCD or TUNEL assays. The average DNA fragmentation rate for each assay was 25 ± 19%, 16 ± 17%, and 8 ± 7%, respectively.

Semen samples from 10 infertile normozoospermic patients (age 41.8 ± 6.1yr) were independently evaluated by the three different assays. The average sperm concentration was 68.5 ± 16.1x10^6/ml with a motility of 53.8 ± 4%, and morphology of 4.6 ± 2%. The assays detected a mean DNA fragmentation rate of 18.1 ± 13% for SCSA, 9.9 ± 8% for SCD, and 7.1 ± 3% for TUNEL. A weak correlation was found between SCSA and SCD (P = 0.05), but neither correlated with the TUNEL method.
Due to the ability of SCD and TUNEL to assess individual spermatozoa, we then assessed a total of 19 samples (mean concentration of 70.6 ± 30.5x10^6/mL, motility of 51.1 ± 17%, morphology of 4.4 ± 2%) by SCD and TUNEL assay. A mean fragmentation rate of 11.1% ± 7 and 6.4% ± 4 was obtained respectively. However, there was no meaningful correlation between them for a given sperm population.

Finally, a concurrent SCD-TUNEL assessment of DNA fragmentation was conducted on semen samples from 10 infertile men with an average age of 40.1 ± 9yrs. These samples had an average concentration of 35.6 ± 2.1x10^6/mL, motility of 53.4% ± 11, and morphology of 4.5% ± 2. Following the individual evaluation of 2224 spermatozoa, 20.4% (n = 455) had fragmented DNA after SCD and 17.3% (n = 384) were also positive for TUNEL, resulting in a strong correlation (r = 0.931, P < 0.001) between the two tests.

Conclusions: The simultaneous assessment of chromatid integrity by the two different assays clearly identified spermatozoa with abnormal DNA. The enhanced correlation may be attributed to the treatment that results in the removal of protamines, thereby allowing a better DNA exposure. Notwithstanding the ability of both assays to detect DNA breakage, the higher sensitivity of SCD requires an adjustment of the thresholds.

P-079 Gene density - a determinant of synaptonemal complex length in human males?

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Introduction: It is well established that correct chromosome pairing and segregation during meiosis is crucial for the generation of haploid gametes in sperm and oocytes. In addition, genetic recombination is critical for both genetic diversity, and to ensure correct chromosome segregation. Recent immunofluorescence techniques have replaced laborious and low-yield conventional cytogenetic studies. Advancing our understanding of the temporal aspects of synaptonemal complex (SC) formation, sex specific differences in recombination, SCs and recombination in infertile males, and the relationship between aneuploidy and recombination. Visualisation of meiotic proteins at the pachytene stage of prophase I stage is made possible by using antibodies against the synaptonemal complex (SC, SCP1 & SCP3), to sites of genetic recombination (MLH1) and centromeres (CREST). Whilst immunofluorescence studies enable complex studies of chromosome pairing and recombination to be performed, they are unable to identify synaptonemal complexes (SCs) of individual chromosomes. The ability to identify individual SCs represents a rich resource of chromosome-specific information, as not all chromosomes behave in the same manner (e.g., individual chromosomes vary in size, GC content, CpG islands, number of genes, length of genes, gene deserts and junctions, single nucleotide polymorphisms and copy number variation, frequencies and sites of recombination). Identification of individual SCs is ultimately more useful to our understanding of human meiosis than current global (per cell) assessments can provide. Little research, has focused on the reliability of identifying individual human synaptonemal complexes (SCs) solely based on physical length alone. Such identification would eliminate the need for complex and costly centromeric multicolour FISH (cenMFISH) experiments to obtain individual SC data. The ability to identify individual SCs and to ascertain chromosome-specific differences in chromosome pairing and recombination should increase our understanding of the behaviour of individual chromosomes and might correlate to many of these chromosomal properties.

Materials and Methods: Immunofluorescence studies and cenMFISH were performed on testicular biopsies from ten donors, identifying 9,900 individual SCs in 450 cells. Following identification of SC number, individual SC lengths were measured using MicroMeasure 3.3.

Results: If SC length correlates to the physical length of the chromosome, we would expect that the SCs corresponding to chromosome numbers 1, 2 and 3, would be the longest, second-longest and third-longest, respectively. However, SC physical length and SC chromosome number rarely correlated. Corresponding SC length and SC number was only observed in 1-7% for chromosomes 8, 13, 17, 18, 19, 21, and 22; 12-22% for chromosomes 4, 5, 6, 7, 9, 10, 11 and 12; 55-82% for chromosomes 1, 2, 3 and 20. However, patterns of variability in SC length were conserved among all donors. SCs for chromosomes 12, 17, 18 and 22 ranked longer than would be expected in 83-99% of cases; SCs for chromosomes 4, 8, 13, 14, 18 and 21 ranked shorter than expected in 72-98% of cases. SC length is not a reliable predictive factor to identify individual SCs, but if SCs are grouped according to size, they can be assigned with ≥95% accuracy into groups for chromosomes 1-2; 3-12; 13-20 and 21-22.

Discussion: Despite extensive variability in SC length, distinct patterns emerged. Principally gene-rich chromosomes had consistently longer SCs and gene-poor chromosomes had shorter SCs, while SC lengths for chromosomes with intermediate gene density were distributed roughly as expected. This suggests that, as observed in mitosis, the degree of chromatin condensation in meiosis is dependent on gene density. These results demonstrate that physical length alone does not allow for detailed individual analysis, but it does...
enable researchers to study data in smaller groups than simply using aggregate autosomal data when costly, complex and time consuming cenM-FISH-based methods are not available.

P-080 Analysis of the acrosome reaction in human sperm using ZP3 chimeric proteins
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Introduction: ZP3 is one of the components of the oocyte extracellular coat (zona pellucida) and it is an in vivo initiator of the acrosome reaction (AR) of human sperm. The precise molecular mechanisms responsible of the induction of the AR mediated by ZP are still unknown. The acrosome reaction process is species specific. Although human ZP3 (hZP3) and mouse ZP3 (mZP3) have an amino acid identities close to 67%, these proteins have a different glycosylation. In the N-terminal region of the mZP3, there is an O-glycosylation cluster that it is not present in the hZP3. While the C-terminal region both mZP3 and hZP3 is glycosylated however it is not identical. To investigate whether the N- and C-terminal domains of hZP3 are involved in the human AR activity, two chimeric proteins between human and mouse ZP3 were created and their activities were evaluated using AR assays.

Materials and Methods: Human ZP3 cDNA (kindly provided by Dr. Dean, NIH, USA) and mouse ZP3 cDNA (Invitrogen) were subcloned in a plasmid pEGFP-N1 (Clontech). Then, two new proteins were subcloned. The first chimeric protein (m-hZP3) was formed by a N-terminal insert of mZP3 (M1-L46) into hZP3. The second chimeric protein (h-mZP3) was formed by a C-terminal insert of mZP3 (F269-Q424) into hZP3. The different constructions were electroporated into CHO cells. Seven different human semen samples were obtained from volunteers. Informed consents were obtained from all subjects participating in the study as approved by the ethics committee at the University of Murcia. The semen samples were selected by swim-up protocol during 1 hour incubation at 37°C and 5% CO₂ atmosphere in HTF medium supplemented with human serum albumin. AR assays were performed using 10 x 10⁶ sperm/ml incubated with four ZP glycoproteins (100µg/ml) secreted by CHO cells and progesterone (10µM). The AR was evaluated using Picosatium lectin conjugated with FITC.

Results: The recombinant glycoproteins hZP3, mZP3, m-hZP3 and h-mZP3 have an apparent molecular weight of 60 kDa, 82 kDa, 64 kDa and 73 kDa respectively. The cell culture supernatant with hZP3, mZP3, and h-mZP3 induced 54, 54.8 and 55% human sperm AR respectively, like the natural agonist of AR. These results suggest that N-terminal region (23-46) and C-terminal region (269-350) of hZP3 are not essential for the induction of the acrosome reaction. This study is supported by a grant from Fundación Séneca de la Región de Murcia 0452/GERM/2006 and IVI-Murcia, Murcia, Spain.

P-082 The involvement of IL-1 family members in the development of experimental autoimmune orchitis
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Introduction: Orchitis is a chronic or acute, symptomatic disease of the testis, often accompanied by local or systemic inflammation within the testes. Progressing orchitis is usually determined by destruction of the testicular structure leading to disturbance of spermatogenesis, germ cell arrest and eventually to male infertility. Cytokines are considered to play a major role in the maintenance of normal testicular function. Under normal conditions members of the Interleukin-1 (IL-1) family are expressed at basal levels in the different types of testicular cells. On the other hand, several cytokines are increased in testes following inflammation. Knock out mice for IL-1Receptor antagonist (IL-1Ra K/O) show an elevation of IL-1α and IL-1β in testicular tissue under normal conditions. The aim of this study is to investigate the expression levels and the cell origin of IL-1 family members (IL-1α, IL-1β and IL-1Ra) in the testes of experimental autoimmune orchitis mice (EAO).

Material and Methods: A new model for murine EAO was established. In brief, testes of adult wild type (WT) BALB/c mice (12 weeks old) were homogenized, mixed with complete Freund’s adjuvant and injected subcutaneously in tail, neck and foot pad of WT and IL-1Ra-K/O mice. Pertussis toxin (20mg/ml) was used as a co-adjuvant. Adjuvant control groups received a similar treatment, by replacing testis homogenate with saline.

At day 70 mice were sacrificed, testes were weighted and either fixed in paraformaldehyde (2%) or frozen in liquid nitrogen. Orchitis was assessed based on examinations of hematoxylin-eosin stained paraffin sections. IL-1β, IL-1Ra and IL-1 receptor type I levels in the testes of mice in different stages of orchitis were measured using specific ELISA methods. The localization of IL-1 in the testis of the EAO and control groups was determined by immunofluorescence. Statistical Analysis was performed by comparing the four groups of magnification between pretreated and post treated mice using the Wilcoxon matched pairs test. Data were analyzed using R software v 2.9.0.

Results: All the differences have been statistically significant except for the magnification grade III (at least one large vacuole, p-value = 0.339). Less vacuolated spermatozoa (grade I and II) showed a significant increase after the antioxidant treatment and the spermatozoa grade IV (with large vacuoles in conjunction with abnormal head shapes or other abnormalities) showed a significant decrease.

Conclusions: This study shows that antioxidant treatment reduces the presence of vacuoles in the spermatozoa nucleus, most probably, because the antioxidant therapy decreases ROS production. In addition, high magnification is a good diagnostic tool that can help allows the identifications of nuclear vacuole-free spermatozoa for assisted reproductive techniques.

P-081 Use of antioxidants decreases the presence of vacuoles in the sperm nucleus determined by high magnification microscopy
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Introduction: Oxidative stress occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the natural antioxidant defenses, resulting in cellular damage. Lifestyle behaviours such as smoking, poor diet, alcohol abuse, obesity or psychological stress; environmental exposure to heat, pollutants and toxins; infections, etc., have all been linked with oxidative stress. Due to the relation between vacuoles and oxidative stress, the embryos derived from a spermatozoa with a high content of vacuoles could involve abortion or implantation failures. Our aim in this study was to assess the efficacy of antioxidants by determining the presence of vacuoles in the spermatozoa head at 8000 x.

Material and Methods: In a prospective study, 33 patients were analyzed, before and after antioxidant treatment, using a high magnification Normarski interferential Leica AM6000 inverted microscopy. The spermatozoa morphology was assessed in vivo at 8000x magnification and spermatozoa were classified into four grades according to the presence and size of the vacuoles (Bar-toov et al., 2003; Hazout et al., 2003; Berkovitz et al., 2006; Vanderzwalmen et al., 2007).

The antioxidant treatment is based on the administration of L-carnitine, oligoelements and vitamins for at least one month. Statistical Analysis was performed by comparing the four grades of magnification between pre and post treated semen and using the Wilcoxon matched pairs test. Data were analyzed using R software v 2.9.0.

Results: All the differences have been statistically significative except for the magnification grade III (at least one large vacuole, p-value = 0.339). Less vacuolated spermatozoa (grade I and II) showed a significant increase after the antioxidant treatment and the spermatozoa grade IV (with large vacuoles in conjunction with abnormal head shapes or other abnormalities) showed a significant decrease.

Conclusions: This study shows that antioxidant treatment reduces the presence of vacuoles in the spermatozoa nucleus, most probably, because the antioxidant therapy decreases ROS production. In addition, high magnification is a good diagnostic tool that can help allows the identifications of nuclear vacuole-free spermatozoa for assisted reproductive techniques.
on the histological data, the disease progression was subdivided into five stages and the animals were grouped accordingly. ELISA results show significant elevation (p < 0.001) in IL-1α, IL-1β and IL-1Ra levels in testicular homogenates of EAO mice compared to control mice. These results were further supported by real-time PCR analysis showing a similar picture. The elevation of the cytokines coincided with the current stage of EAO in mice.

Compared to the control group the testes of WT-EAO mice showed a massive expression of IL-1 family members, mainly in the interstitial tissue, which was often infiltrated by leukocytes. Remaining cells of the seminiferous tubules expressed IL-1 members at lower levels compared to interstitial cells. Immunofluorescence data revealed that all expression levels of the IL-1 family were elevated in EAO mice compared to those of control animals. At day 50 in the EAO-IL-1Ra K/O group 94% of mice developed orchitis, whereas 50% of WT-EAO mice developed orchitis, as testicular weight and histo-scores revealed. The ELISA and realtime PCR examinations for the EAO-IL-1Ra K/O group showed a drastic elevation in IL-1 expression in the testis homogenate which exceeded IL-1 levels of the EAO WT group.

Conclusion: Members of IL-1 family are involved in the development of experimental autoimmune orchitis and might play a crucial role in the onset and progression of the disease. Further investigations are required to determine their distinct role within the cytokine network of the testis under physiological and pathological conditions and their possible contribution to future treatments of male infertility.

P-084 Is morphologically selected sperm injection useful for patients selected for teratozoospermia or for previous implantation failures?

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Introduction: Morphological selection of fertilizing spermatozoa under a high magnification (IMSI) has been introduced to provide a better clinical outcome than the conventional intracytoplasmic sperm injection (ICSI). However, the relevance of this technique is not yet well evaluated, since no study has clearly shown which infertility indications among male factor or implantation failure would benefit the most from IMSI. Therefore, the aim of this study was to perform IMSI including selected patients for either (i) strong teratozoospermia or (ii) previous ICSI implantation failures without strong teratozoospermia, to evaluate if it could improve the ongoing pregnancy rate (OPR) compared to conventional ICSI.

Patients and Methods: From October 2007 to October 2009, we prospectively included 340 infertile couples undergoing IVF with micromanipulation treatment. Two male infertility indications were enrolled for either IMSI or ICSI procedures. First, the teratozoospermia (T) group was defined by the presence of a severe male factor (typical spermatozoa with WHO criteria < 10% in fresh ejaculated semen and < 10% in selected sperm) associated with < 2 previous ICSI failures. Second, the implantation failure (IF) group was defined by ≥ 2 previous good quality embryo transfers with no pregnancy. In IF group, sperm inclusion criteria were typical spermatozoa > 10% in fresh ejaculated semen and > 20% in selected sperm. IMSI procedure was based on a preliminary motile sperm organelle morphology examination and choice under x6000 high magnification followed by conventional ICSI. Embryo transfers were performed at day 3. The primary end point was OPR (intra-uterine pregnancy older than 12 weeks of amenorrhea) compared in IMSI and ICSI groups for T and IF groups respectively.

Results: In all, 175 ICSI and 165 IMSI cycles were analysed, with 88 IMSI compared with 101 ICSI in the IF group, and 77 ICSI with 74 ICSI in the T group. OPR was not different after IMSI compared to ICSI in the IF group (18.2% (16/88) vs. 18.8% (19/101); p = 0.91). However, we observed a significant higher OPR after IMSI compared to ICSI in the T group (40.3 (31/77) vs. 17.6% (13/74); p = 0.002).

Conclusion: Our study suggested that the IMSI procedure is a valuable option for patients with a strong teratozoospermia at their first or second attempts, but not for patients with repeated previous ICSI failures in the absence of a severe male factor.

P-085 Activity of glutathione peroxidase in seminal plasma and its relationship with standard semen parameters and cigarette smoking

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Introduction: Oxidative damage is one threat spermatozoa have to face during epididymal maturation and storage. However, beside their noxious effects, a small and controlled amount of reactive oxygen species (ROS) is needed for sperm physiology in processes such as sperm maturation and capacitation. It is therefore essential a balance between ROS production and its elimination in the sperm cell surroundings. Seminal plasma contains both non-enzymatic and enzymatic ROS scavengers such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). Among the different antioxidant enzymes, GPX occupies a peculiar position. This enzyme uses different substrates in addition to H2O2 and functions even for small variations of their concentrations.

The aim of this study is to correlate the oxidative stress defence in terms of GPX activity to the WHO (World Health Organization, 1999) parameters of sperm density, morphology and motility, and to tobacco consumption.

Materials and Methods: The population under study consisted on 275 consecutive males from infertile couples subjected to IVF/ICSI program in our Human Reproduction Unit from Cruces Hospital (Vizcaya, Spain). All semen samples were collected by masturbation following 3 days of abstinence and analyzed according to WHO criteria. Both motility and morphology parameters were organized into three groups according to (a) progressive motility (normal or ≥ 50%, moderate asthenozoospermia or < 50% and > 20%, and severe asthenozoospermia or < 20%), and (b) normal morphology (normal or > 14%, moderate teratozoospermia or ≤ 14% and > 4%, and severe teratozoospermia or ≤ 4%). Total GPX activity was spectrophotometrically determined using cumene hydroperoxide as substrate and data were analysed with SPSS 16.0 statistical package.
Introduction: During semen evaluation, cryptozoospermia is defined by the absence of spermatozoa in the counting chamber (less than 10^6 spermatozoa/ml) but with the presence of some sperm in the pellet of centrifugation of the whole ejaculate. This situation is characterized by a sperm count fluctuation which may result in the lack of motile sperm for ICSI on the day of oocytes retrieval. To avoid this situation, we proposed to patients, with transient azoospermia or repeatedly cryptozoospermia, the constitution of a safety pool of frozen spermatozoa, before ICSI attempts. The frozen sperm are used only when fresh sperm are not available for ICSI. This study evaluates our 4 years’ practice of such a cryptozoospermia management, comparing ICSI results obtained with ejaculated fresh sperm and with frozen spermatozoa.

Material and Methods: This study concerns 206 patients with cryptozoospermia (285 ICSI attempts). A subgroup of 68 patients (83 attempts) with extremely poor sperm is distinguished. ICSI results from cryptozoospermic patients were compared with those obtained in our ART center for the same period, by ICSI with fresh sperm (>10^6/ml) and with testis frozen sperm from non obstructive azoospermia. The studied parameters are the fertilization rate, the cleavage rate, the proportion of good quality embryos (transferred and frozen at day3) the pregnancy rate and the delivery rate (calculated for the first 3 years) per oocytes pick-up.

Results: From January 2006 to December 2009, 125 out of the 206 men presenting cryptozoospermia constituted a stock of frozen ejaculated sperm. For 42 of them (55 ICSI), frozen sperm have to be used during the ICSI attempts.

- The proportion of weak oocytes recovery (< 4 metaphase II oocytes) is similar in all groups according with female’s age.
- The fertilization rate is significantly lower using frozen ejaculated sperm (48.8%) than using fresh sperm (73.5%, p < 0.005) or testis frozen sperm (71.5%, p < 0.005).
- The cleavage rate is similar between all groups.

- Less than 55% of the embryos are of good quality at day 3 in the subgroup of 83 ICSI with extremely low sperm count. This rate of good quality embryos is comparable in the other cases of cryptozoospermia and when using testicular frozen sperm (mean 67%).

- The pregnancy rate and delivery rate per oocytes pick-up are similar for the cryptozoospermia group and all ICSI in the centre for the same period (respectively: 28.1%, and 23.4%). The delivery rate appears to be lower in case of extremely poor quality ejaculated sperm (14.6% versus 23.4%, NS) while testicular frozen sperm ICSI from non obstructive azoospermia show a satisfying delivery rate (25.7%, NS).

Conclusion: This study highlights the interest of banking ejaculated sperm in case of cryptozoospermia, avoiding for these patients the recourse to an invasive procedure: Among 20% of cryptozoospermia men present an azoospermia the day of the ICSI, leading to the use of frozen ejaculated sperm.

Even if the fertilization and good quality embryo rates are decreased, the pregnancy rate remains good. This study illustrates the difficulty to obtain a healthy child by ICSI when spermatogenesis is disturbed: The delivery rate seems to be linked with the severity of the oligozoospermia, pointing out a correlation between quality and quantity of ejaculated sperm.

The comparison of results obtained with ejaculated extremely poor sperm and with testis frozen sperm suggests that, in cryptozoospermia management, the testicular sperm extraction could conserve an interest.

P-088 Comparison between sperm DNA fragmentation and OxyDNA in samples with severe oligoasthenoteratozoospermia

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Introduction: It has been shown that DNA fragmentation is a marker of sperm quality and a potential fertility predictor. DNA damage in human spermatozoa has been associated with a range of adverse clinical outcomes including infertility, abortion, and disease in the offspring. It is well known that poor chromatin packaging and impaired antioxidant capacity are associated with DNA damage. This damage involving impaired chromatin remodelling during spermiogenesis is followed by a free radical attack to induce DNA strand breakage.

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It has recently been demonstrated that 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG) positive spermatozoa (overload of reactive oxygen species) are associated with decreased sperm motility and lower embryo quality after IVF or intracytoplasmic sperm injection (ICSI). Our study aims to evaluate if remodelling of chromatin and an impairment of DNA protonation during late spermatogenesis is correlated with DNA fragmentation.

**Materials and Method:** A total of 84 sperm samples with severe oligoasthenoteratozoospermia (OAT) were collected and analyzed. Sample’s parameters, such as viscosity, volume, concentration, motility and morphology, were evaluated according to the WHO guidelines. The semen samples were processed for DNA fragmentation by TUNEL using the commercially available in-situ Cell Death Detection Kit (Roche) and for sperm DNA oxidation was determined using a fluorescent assay OxyDNA test (Calbiochem) for the detection of 8-oxoguanine as a biomarker for oxidative stress. Oxidation was also evaluated on the seminal line. The positive control sperm cells were incubated for 1 h at room temperature with H2O2 (30% vol). Correlation between TUNEL and OxyDNA was estimated through the Pearson correlation coefficient. Differences between medians were tested through the Mann-Whitney U test. Level of significance was P < 0.05.

**Results:** The median TUNEL percentage measured in the 84 semen samples was 40% (range 1-40) whereas the median OxyDNA percentage was 50% (range 1-35). TUNEL and OxyDNA were directly correlated (r = 0.326; P = 0.02). When limiting the analysis to the 15 samples with increased viscosity this correlation was stronger (r = 0.631; P = 0.012). Finally the median OxyDNA percentage was higher in samples with increased viscosity (69 samples) compared with those with normal viscosity although the difference was not statistically significant (normal viscosity = 5%; increased viscosity = 10%; P = 0.190).

**Conclusions:** The present study suggests a link between DNA fragmentation and oxidative base damage, possibly caused by ROS effect on DNA. OxyDNA may be considered as a biomarker of oxidative damage in DNA, although 8-oxo-dG is a primary oxidation product in DNA by ROS. Moreover we detected a trend towards an increase in oxidative stress in samples with increased viscosity. Although further studies are needed to confirm these results, the technical procedure to assess oxidative base damage combined with sperm DNA fragmentation might be an important tool to explore the origins of alterations of DNA integrity. These findings may also help to improve the clinical management of patients presented with defined male-factor infertility.

**P-089 The sperm motility index after swim-up can be used to predict the occurrence of normal fertilization following conventional insemination during ART**

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**Introduction:** The technology required to analyze the concentration, motility and velocity of sperm during swim-up is available by computer to aid in assisted reproductive technology (ART) treatment. Through use of the Sperm Quality Analyzer-Y (SQA-Y), the evaluation of sperm has become easy and stable. The Sperm Motility index (SMI) which is the original index defined by the company that developed this technology reflects both motile sperm concentration and its velocity. Recent reports have mentioned the SMI as having a positive effect on the rate of fertilization. But no cut-off value has been determined using a fluorescent assay OxyDNA test (Calbiochem) for the detection of 8-oxoguanine as a biomarker for oxidative stress. Oxidation was also evaluated on the seminal line. The positive control sperm cells were incubated for 1 h at room temperature with H2O2 (30% vol). Correlation between TUNEL and OxyDNA was estimated through the Pearson correlation coefficient. Differences between medians were tested through the Mann-Whitney U test. Level of significance was P < 0.05.

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**Conclusions:** The present study suggests a link between DNA fragmentation and oxidative base damage, possibly caused by ROS effect on DNA. OxyDNA may be considered as a biomarker of oxidative damage in DNA, although 8-oxo-dG is a primary oxidation product in DNA by ROS. Moreover we detected a trend towards an increase in oxidative stress in samples with increased viscosity. Although further studies are needed to confirm these results, the technical procedure to assess oxidative base damage combined with sperm DNA fragmentation might be an important tool to explore the origins of alterations of DNA integrity. These findings may also help to improve the clinical management of patients presented with defined male-factor infertility.

**P-090 SPRASA antibodies, a cause of “unexplained” infertility**

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**Introduction:** Despite extensive clinical investigations the cause of infertility remains unexplained for many infertile couples and consequently they may not receive optimal treatment. Recently, we discovered the sperm protein SPRASA as the antigen for antisperrin antibodies in some infertile men. More recently we have found that SPRASA is expressed not only in sperm but also in oocytes, where it is present on the oolemmal membrane and in the zona pellucida. We undertook the following studies to investigate the function of SPRASA and its relationship with infertility/fertility.

**Materials and Methods:** Ethical approval for this study was obtained from the Northern Regional Ethics Committee and University of Auckland Animal Ethics Committee, New Zealand. Sera were obtained from 102 infertile women and their partners attending two fertility services in Auckland, New Zealand. Sera were also obtained from 104 control fertile women and their partners recruited through the post-natal wards of a public hospital and from community volunteers following advertisements in the media. These sera were screened for the presence of IgG antiSPRASA antibodies by an ELISA developed in our laboratory which employed recombinant SPRASA as the antigen.

Two groups of five female CD1 mice were immunised, up to four times, subcutaneously with recombinant human SPRASA or with keyhole limpet haemocyanin (KLH, controls). After the immunisation protocol was complete the females were screened for the stage of their oestrous cycle by vaginal smear or oestrous cycle monitor, then date-mated with male CD-1 mice of proven fertility and monitored for coital plugs. Weights were monitored for 12 days post mating to confirm pregnancy or lack of pregnancy. Mice that were not pregnant were again dated-mated. Mice that were pregnant were euthanised and their litters examined.

**Results:** Three infertile women had markedly elevated levels (>2.2 normalised units) of SPRASA antibodies while none of the fertile women had these levels of antibodies. However, on a population basis the difference in the levels of SPRASA antibodies between infertile and fertile women approached but did not reach statistical significance (P = 0.06). None of the fertile or infertile men had significantly elevated levels of SPRASA antibodies.

All of the control KLH immunised female mice became pregnant within two matings. In contrast, none of the SPRASA immunised females became pregnant despite being mated no fewer than five times each.

**Conclusions:** Antisperrin (and antioocyte) antibodies have long been known to be associated with infertility but these antibodies may also be found in fertile persons, limiting the value of antisperm antibody testing. This is in part because current tests do not distinguish between sperm/ovarian antigens that are crucial for fertility and those that are not. Our results show SPRASA appears to be an antigen that is crucial for fertility in both humans and mice. Although only 3% of infertile women had significantly elevated levels of SPRASA antibodies, female mice immunised with SPRASA were profoundly infertile. This suggests that in a subset of women, SPRASA antibodies may be an important cause of their infertility. The ELISA to detect SPRASA antibodies is a simple and cheap diagnostic test. Screening infertile women for the presence of elevated levels of
SPRASA antibodies may be useful for determining the cause of their infertility and for informing treatment decisions.

P-092 A multi centre evaluation of the new Beckman Coulter anti Müllerian immunoassay (Active AMH Gen II)
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Introduction: Anti Müllerian Hormone (AMH) is produced by human ovarian granulosa cells from early stages of development until the early adult stage. Circulating AMH concentrations in females are reduced from birth until puberty, and then increase rapidly to maximal concentrations. Thereafter, a steady and unrelenting decline is seen until AMH becomes immeasurable in the circulation prior to the menopause. Profiles of the decline in AMH through reproductive life appear to mirror the decay in the number of non-growing primordial follicles – the ovarian reserve. Correspondingly, one of the main clinical utilities of the assay is in the assessment of ovarian reserve, which allows prediction of likely response to control ovarian stimulation (COS).

Until recently two enzyme linked immunosorbent (ELISA) assays have been available; from DSL and Immunotech. These have been standardized differently and give different values for quantitation of AMH. This has created some confusion for investigators. Following the acquisition of DSL and Immunotech by Beckman Coulter the two assays have been harmonized by replacing the DSL ELISA with the Active AMH Gen II assay incorporating DSL antibodies but calibrated using the same standard as used in the Immunotech assay.

The objective of this study was to assess the performance of the new Active AMH Gen II assay versus the original Diagnostics Systems Laboratories (DSL) ELISA assay and make recommendations regarding appropriate clinical cut off values for use in controlled ovarian stimulation (COS) programmes.

Methods: An evaluation of the Active AMH Gen II assay was performed at three sites, each with extensive experience in measuring circulating AMH in the adult human female. Results were compared with the original DSL ELISA assay. A total of 300 patient samples were analysed in duplicate (100 at each of the three sites) to provide comparative data, with additional comparison of a series of common pooled samples at critical clinical levels. Linearity, precision and sensitivity of the new assay were also assessed.

Results: The values obtained demonstrated good agreement with a regression equation AMH Gen II = 1.41DSL – 0.78 pmol/L, r = 0.97, n = 300. Linearity was good with observed values close to expected (mean recovery 106.3%). Within and between batch precision was assessed at various levels on a range of control materials, between batch CVs were (11.1–14.8% at 1.5 pmol/L, 10.1 to 17.7% at 5 pmol/L, 3.4 to 7.9% at 15 pmol/L and 2.9 to 6.8% at 70 pmol/L). The analytical sensitivity of the assay was 0.6 pmol/L.

Conclusions: This study effectively demonstrates that the results from Active AMH Gen II assay correlate well with the original DSL ELISA assay. Laboratories transitioning between methods should expect similar precision and excellent correlation compared to the DSL assay. Values are, however, approximately 40% higher by Gen II so laboratories should adjust their cut points accordingly.