

sperm parameter (count, motility, morphology or combination), the remaining 36 patients (group II) had normal semen analysis. Sperm DNA damage was assessed by the TUNEL assay using epifluorescence (normal <20%) and cleaved caspase-3 concentration visualized by immunocytochemistry (Kotwicka 2008, normal <11%) in both groups and compared. Results are expressed as mean  $\pm$  SD. Statistical analysis was done by student's t-test and Pearson chi-square, a  $p < 0.05$  was considered significant.

**RESULTS:** The mean TUNEL value in group I was  $19.98 \pm 10.7\%$  which was not statistically different than  $18.6 \pm 11.0\%$  in group II ( $p = .480$ ). The mean caspase-3 value was  $8.6 \pm 6.4\%$  similar to the values of  $9.1 \pm 7.4\%$  in group II ( $p = .672$ ). The percentage of patients with abnormal levels of DNA fragmentation were similar in both groups, group I, 52.1%, group II 52.8% ( $p = .940$ ). High levels of caspase-3 were also similar, group I 31.7%, group II 27.8% ( $p = .682$ ).

**CONCLUSION:** Our results showed that infertile men with normal semen analysis and history of poor fertilization or poor embryo quality in vitro, should be tested for sperm DNA fragmentation, since abnormal levels are as common as in patients with poor semen quality. Abnormal sperm DNA integrity can be detrimental to pregnancy outcome and future efforts should be directed toward reducing the percentage of apoptotic sperm in semen samples prior to performing ICSI (Rawe, 2010).

**P-492** Wednesday, October 27, 2010

**ANALYSIS OF HUMAN SPERM QUALITY DURING THE LAST 5 YEARS IN A SPERM DONATION PROGRAMME.** C. Gonzalez-Ravina, T. de Ruz, A. Pellicer, N. Prados, M. Fernandez-Sanchez. Laboratory of Andrology, IVI Sevilla, Sevilla, Spain; Medical-Gynecologist, IVI Valencia, Valencia, Spain.

**OBJECTIVE:** The quality of the sperm parameters seems to have diminished during the last years. The purpose of this study was to examine if these results were observed in our group of young candidates for sperm donation during the last years.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** A retrospective study was conducted at our private clinic involving a total of 625 men from January 2005 to December 2009. These men were healthy sperm donor volunteers with a mean age of 24.5 years (range 18-35). Only the first sperm sample of each candidate was used for this analysis. Concentration, progressive motility and total volume of the samples were recorded following WHO standard procedures. Candidates with less than 110 million of progressive motile sperm in the total of the ejaculate were discarded as donors.

**RESULTS:** We found that 27.8% of the candidates scored under normal WHO values. Another 59.0% had over 20 million sperm/ml and 50% of progressive motility but less than 110 million sperm and were also discarded. Only 13.2% of the total volunteers were accepted in the program (95%CI; 10.5-15.8). No difference was found in the rate of acceptance between the different 5 years ( $p = 0.90$ ). The mean volume (2.7 ml; 95% CI 2.6-2.8), sperm concentration (41.0 millions/ml; 95% CI 38.9-43.1) and rate of progressive motility (62.3%; 95% CI 61.1-63.6) also did not vary during the 5 years ( $p = 0.10$ ; 0.73; 0.49).

**CONCLUSION:** In summary, we did not find any relationship between the year of study and the sperm parameters. Our rate of acceptance is similar to other programmes. Further analysis over more years will be necessary to value any impact on the sperm quality of this population.

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**EFFECT OF ANTIOXIDANT AND OXIDATIVE STRESS ON THE OUTCOME OF INTRACYTOPLASMIC SPERM INJECTION CYCLES.** M. Nichi, D. Braga, A. Setti, P. Goes, A. Dalmazzo, E. Borges, Jr. Animal Reproduction Department, University of Sao Paulo, Sao Paulo, Brazil; Fertility - Assisted Fertilization Center, Sao Paulo, Brazil; Sapientiae Institute - Educational and Research Center in Assisted Reproduction, Sao Paulo, Brazil.

**OBJECTIVE:** Oxidative stress is one of the most important causes of male infertility. However, the influence of seminal oxidative status when using intracytoplasmic sperm injection (ICSI) is still a matter of debate. This study was designed to evaluate the effect of the antioxidants catalase (CAT) and glutathione peroxidase (GPx), and the oxidative stress marker malondialdehyde (MDA) in semen samples submitted to ICSI cycles on reproductive outcomes.

**DESIGN:** Prospective.

**MATERIALS AND METHODS:** Semen samples of 66 men were collected by masturbation; an aliquot used for ICSI and another aliquot centrifuged and the supernatant used to measure MDA levels and CAT and GPx

activities by spectrophotometry. Student's T-test was used, and logistic models were calculated using TBARS levels and CAT and GPx activity as independent variables and the occurrence of pregnancy as a binary dependent variable. Also, a correlation analysis was performed in order to verify the relationship between response variables.

**RESULTS:** No difference was found on seminal activity of GPx when comparing men in which ICSI cycles resulted on pregnancy with those that did not achieve pregnancy (Pregnant:  $87.6 \pm 13.45$  vs. Non-pregnant:  $73.7 \pm 9.0$  UI/mL;  $p = 0.13$ ). However, a negative correlation was found between GPx and progressive motility ( $r = -0.23$ ,  $p < 0.05$ ). On the other hand, MDA levels were higher in the previous group (Pregnant:  $204.5 \pm 10.5$  vs. Non-pregnant:  $177.6 \pm 7.36$  ng/mL;  $p = 0.035$ ). Furthermore, odds of pregnancy were higher with the increase on MDA levels (OR: 1.012; CI: 1.0-1.023;  $p < 0.04$ ). Also, positive correlations were found between MDA levels and implantation rates and the proportion of high quality embryos ( $r = 0.25$  and  $r = 0.23$ , respectively;  $p < 0.05$ ).

**CONCLUSION:** We concluded that GPx may be a marker of poor semen quality. Also, the presence of moderate levels of oxidative stress in semen of men submitted to ICSI may be beneficial to the reproductive outcomes, probably due to the role of the reactive oxygen species on reproductive physiology.

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**LONG TERM FOLLOW-UP OF A RANDOMIZED CONTROLLED TRIAL ON THE EFFECT ON VARICOCELECTOMY.** Z. Brown, C. J. G. Wensing, W. P. Th. M. Mali, E. R. te Velde, H. C. Romijn, J. S. E. Laven. Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus Medical Centre, Rotterdam, Zuid-Holland, Netherlands; Department of Radiology, University Medical Centre Utrecht, Utrecht, Netherlands; Department of Public Health, Erasmus Medical Centre, Rotterdam, Zuid-Holland, Netherlands; Faculty of Veterinary Sciences, University Medical Centre Utrecht, Utrecht, Netherlands.

**OBJECTIVE:** To assess time to pregnancy (TTP) as a proxy of fertility, endocrine status, testis volume and semen parameters in adult men who had either a varicocelectomy at adolescence or were left untreated.

**DESIGN:** Assessment at intake and at 1, 3 and 20 years of follow-up in a randomized controlled trial.

**MATERIALS AND METHODS:** Participants with a varicocele were randomly assigned into either a treated (1) or untreated group (2). Healthy volunteers were assigned to group 3 (control). At 20 years all participants received a questionnaire assessing TTP and were invited to visit the outpatient clinic to have a standardized clinical and endocrine examination as well as a semen analysis. Comparisons were made between and within all groups using non-parametric test.

**RESULTS:** A total of 64 participants (72.7%) responded to the questionnaire, 34 (53.1%) of them visited the outpatient clinic. The mean TTP was 3.2 months in group 1 (range 1-12), 2.6 months in group 2 (range 1-15, NS compared with group 1) and 6.7 months in group 3 (range 1-50,  $p < 0.01$  compared with group 1 and 2). Moreover, there were no differences in the number of children between different groups. Differences in testis volumes after 20 years were not significant between and within all groups. There were no differences between the groups concerning serum follicle stimulating hormone, luteinizing hormone and testosterone levels. Inhibin B levels in group 1 and 2 were lower at adult age compared with group 3 ( $p < 0.05$ ). Semen parameters did improve initially in the treated group compared to controls and untreated individuals. However, semen concentration as well as motility and morphology were all well within the normal range in untreated and treated individuals and were all comparable to those seen in controls.

**CONCLUSION:** Although treatment of varicocele during adolescence seems to improve semen quality initially it did not have any long term effect on either fertility or fecundity.

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**EFFECT OF DIETARY DHA SUPPLEMENTATION ON SPERM DNA INTEGRITY.** J. C. Martinez-Soto, J. C. Domingo, B. Cordobilla, L. Palbero, A. Pellicer, J. Landeras. IVI Murcia, Murcia, Spain; Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain; IVI Valencia, Valencia, Spain.

**OBJECTIVE:** Oxidative stress is an imbalance between the amount of reactive oxygen species (ROS) produced and the ability of the antioxidants

to scavenge these. High levels of ROS produce DNA damage which could cause infertility. Docosahexaenoic acid (DHA) is a polyunsaturated Omega-3 fatty acid and is found in high concentrations in sperm membranes. Several studies suggest an antioxidant capacity for DHA. The aim of this study is to evaluate the effect of Brudy Plus® an enzymatic nutraceutical triglyceride oil with a high concentration of DHA in damaged spermatozoa DNA.

DESIGN: A randomised double-blind study with a control group.

MATERIALS AND METHODS: 46 male patients were included in the present study. DHA group A (n=21) was given a supplement of 1050mg/day Brudy Plus® for 10 weeks. The placebo group B (n=15) was given a supplement of 1050mg/day of sunflower oil for 10 weeks. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining was used to determine sperm DNA fragmentation. The total antioxidant capacity of seminal plasma was measured using myoglobin, which interacts with 2,2-azino-bis(3-ethylbenzo-thiazoline-6-sulphonate) (ABTS). The latency phase (Lag) in the accumulation of ABTS cation is proportional to antioxidant concentration.

RESULTS: In group A, there was a statistically significant decrease in DNA fragmentation which is directly proportional to the number of weeks of treatment (0 weeks 25.98 ± 4.73, 5 weeks 15.60 ± 2.46, 10 weeks 8.79 ± 1.92) P < 0.01.

In group B, DNA fragmentation was not affected by treatment (0 weeks 17.81 ± 2.56, 5 weeks 23.50 ± 4.55, 10 weeks 29.01 ± 6.13) P = 0.25.

No significant variations in the level of DHA were observed in the spermatozoa membrane or in the seminal plasma. Lag showed significantly greater values in group A patients vs group B patients.

CONCLUSION: The results show that dietary DHA supplementation decreases sperm DNA damage and improves the antioxidant system. Therefore Brudy plus® can be used as a therapy of choice for sperm with high DNA fragmentation.

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**P-496** Wednesday, October 27, 2010

**RELATIONSHIP BETWEEN SEMEN PARAMETERS AND OUTCOME OF INTRAUTERINE INSEMINATION (IUI).** J. Biggs,

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OBJECTIVE: The clinical relevance of the various parameters measured in routine semen analysis is not well defined. The study objective was to evaluate outcomes of IUI according to sperm concentration, motility, post-wash total motile sperm (TMS), progression and abnormal morphology.

DESIGN: Retrospective review.

MATERIALS AND METHODS: Records of IUI cycles performed during 2004-09 were reviewed. Inclusion criteria were female age <43 years, diagnosis of unexplained infertility and stimulation using a standard CC/FSH protocol. Analysis was restricted to the first attempt per couple during the review period. HCG (10,000 IU) was administered when there was at least one follicle >17 mm, followed 36 hours later by a single insemination with a 0.3 ml post-wash semen sample. Clinical pregnancy rate (gestational sac) was evaluated according to post-wash semen parameters (concentration, motility, TMS, and progression) of the sample used for insemination by logistic regression. For a subset of couples, percent abnormal morphology from an initial diagnostic semen analysis was also related to pregnancy outcomes by logistic regression.

RESULTS: A total of 2410 couples met the inclusion criteria. Pregnancy outcomes were not significantly related to either progression (p=0.64) or sperm morphology abnormalities (p=0.15, n=1326). Pregnancy rates increased significantly with increasing TMS <8 million (p<0.0001). However, there was no apparent relationship between pregnancy and TMS ≥ 7 million (p=0.28). Among patients with ≥ 7 million TMS, neither concentration (p=0.59) nor motility (p=0.44) had any apparent effect on pregnancy rates.

Total motile sperm	<2 million	≥2, <4 million	≥4, <7 million	≥7 million
Cycles	57	104	220	2029
Pregnancy (%)	0	9.6	14.1	17.4

CONCLUSION: Of all the parameters measured in routine semen analysis, the only parameter that was significantly related to IUI pregnancy

outcomes was TMS. Outcomes were stable with TMS >7 million, but declined gradually with decreasing counts below 7 million.

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**DECLINING QUALITY ON SEMEN COLLECTED FOR ICSI CYCLE WHEN COMPARED TO PREVIOUS SEMEN ANALYSIS COLLECTED FOR INFERTILITY EVALUATION.** F. Pasqualotto, E. Borges, Jr, D. Braga, R. Ferreira, A. Iaconelli, Jr, E. Pasqualotto. University of Caxias do Sul, Caxias do Sul, RS, Brazil; Fertility - Assisted Fertilization Center, Sao Paulo, SP, Brazil; Sapientiae Insitute - Educational and Research Center in Assisted Reproduction, Sao Paulo, SP, Brazil.

OBJECTIVE: The aim of this study was to investigate possible differences in semen quality when samples were collected to be used on intracytoplasmic sperm injection (ICSI) cycles and samples collected for simple infertility evaluation purposes.

DESIGN: Retrospective.

MATERIALS AND METHODS: Semen samples were obtained from 617 male partners of women submitted for ICSI cycles treatment between January 2006 and December 2009. All samples were collected in the same room and evaluated by the same person. Semen specimens were collected after 3-5 days of abstinence and were analyzed for sperm concentration and percent progressive motility according to World Health Organization criteria and sperm morphology according to Tygerberg's strict criteria. Differences in semen parameters between sperm samples collected for instant use in ICSI cycles compared to previous sperm analysis in the same man were analyzed statistically.

RESULTS: Sperm concentration was significantly lower (p= 0.03) in semen samples collected on the day of oocyte retrieval (17.2 ± 5.02) compared to those samples provided for previous seminal analysis for infertility evaluation (29.2 ± 9.1). Also, sperm motility decreased from the day of infertility investigation (52.7 ± 21.8) to the day of oocyte retrieval (31.7 ± 12.9; p = 0.04). On the other hand, no differences were found on sperm morphology according to the Tygerberg's strict criteria between the day of infertility investigation (5.1 ± 1.8) and the day of oocyte retrieval (4.3 ± 2.7; p= 0.08).

CONCLUSION: Our results suggest that the decrease in semen quality is at least partly result of the acute psychological stress that is experienced by the patients who are requested to provide a semen sample at the day of in vitro fertilization. Due to this decrease in semen quality, semen cryopreservation may be suggested for men with severe oligospermia to overcome azoospermia/severe oligospermia on the day of ICSI procedure.

**P-498** Wednesday, October 27, 2010

**IMPROVED PREGNANCY RATES IN MALE FACTOR BY HYALURONIC ACID BINDING SPERM SELECTION AND MSOME IN ART.** S. Cubillos, S. Sanchez, J. Pedraza, F. Caldiño, S. Cuneo. Laboratorio de Reproduccion Asistida SA de CV, Concibe Reproduccion Asistida, Mexico, DF, Mexico; Clinica de Reproduccion Asistida SA de CV, Concibe Reproduccion Asistida, Mexico, DF, Mexico.

OBJECTIVE: Implantation and Pregnancy rates after ICSI is positive correlated with morphological nuclear normality of spermatozoa. 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. Spermatozoa have a specific affinity to bind HA (hyaluronic acid) a natural protein found in the cumulus complex surrounding the oocyte. 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.

DESIGN: Retrospective and Descriptive study.

MATERIALS AND METHODS: Study from august 2009 to January 2010 of 40 patients with female ages between 30 and 38 years old, with severe male factor who underwent 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). Mann-Whitney U-tests were carried out. Significance was defined as p < 0.05.

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.