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concentrations above and below 15 million/ml, respectively). Serum concentrations of FSH, LH, estradiol, testosterone and prolactin, and the sperm parameters were compared between the three groups of genotypes (GG, GA and AA). Furthermore, the frequencies of these three genotypes were compared between cases and controls.

Main results and the role of chance: This is the first study that investigated the SNP rs 175080 in Caucasian men. There were no significant differences in anthropometric parameters and hormonal values between the three groups of genotypes. A significantly lower sperm concentration was found in men with the AA genotype as compared to men with the GG and GA genotypes ($p < 0.001$). The group with the AA genotype had the lower progressive motility values as compared to the two other groups ($p < 0.05$). Also, there was a significantly different distribution of the frequencies of the three genotypes between cases and controls ($p < 0.001$).

Limitations, reason for caution: The relatively small sample size of the present study does not allow drawing solid conclusions. Besides, our results refer to Caucasians, therefore, these conclusions should not be expanded.

Wider implications of the findings: Polymorphisms may be widely used for the investigation of male infertility. The present study provides data for further research in the context of larger studies.

Study funding/competing interest(s): Funding by University(ies) – University of Thessaly, Department of Obstetrics and Gynaecology.

Trial registration number: NA.

Keywords: MLH3, polymorphism, male infertility

P-071 A combination of eight micronutrients is superior to a mono preparation comparing improvement of variant groups of impaired sperm motility

F. Bodner¹, M. Lipovac², M. Imhof²

¹Landeskrankenhaus Wienviertel Korneuburg, Korneuburg, Austria

²Landeskrankenhaus Wienviertel Korneuburg, Gynaecology, Korneuburg, Austria

Study question: The objective of this retrospective study was to compare the effect of a combination of 8 active compounds (l-carnitine 440 mg, l-arginine 250 mg, zinc 40 mg, vitamin E 120 mg, glutathione 80 mg, selenium 60 µg, coenzyme Q10 15 mg, folic acid 800 µg) with a single active compound (l-carnitine 1000 mg) on subgroups of impaired semen motility.

Summary answer: Both therapies increased semen motility to a significant level. A low previous fraction of fast and slowly progressive sperm results in a higher improved by means of micronutrient supplementation. The combination supplement was significantly superior in improvement of progressive and total motility.

What is known already: Approximately 50% of all infertile couples' cases are related to an impaired semen quality which is caused in 30–80% by oxidative stress. Oral intake of micronutrients can ameliorate sperm motility

Study design, size, duration: From 2006 to 2014 261 patients of the IMI Fertility Center, Vienna and the Med19 Study Center, Vienna were enrolled in the study. For 3 months patients took either the mono preparation or the combination treatment. Semen analysis was performed before and after intervention. Motility subgroups were statistically.

Participants/materials, setting, methods: Patients were 18–60 years old, suffered from subfertility over 1 year, had one or more recent pathologic semen analysis according WHO 2010 and didn't meet any exclusion criteria. 144 subfertile men were treated with a mono preparation and 127 patients received the combined preparation.

Main results and the role of chance: Within both therapy groups, treatment regimes increased semen motility to a highly significant level ($p < 0,001$). Subgroup analysis revealed that the lower the previous fraction of fast and slowly progressive sperm has been, the greater the improvement was. Motility counts of 40% progressive sperms and more couldn't be increased significantly any further. The combined therapy increased rapidly progressive motile sperms highly significantly ($p = 0,004$) and overall progressive sperm count significantly ($p = 0,01$) compared to the mono-substance group.

Limitations, reason for caution: –

Wider implications of the findings: –

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Lenus Pharma, Seeböckgasse 59, 1160 Vienna, Austria.

Trial registration number: NA.

Keywords: sperm, oxidative stress, motility

P-072 Mycoplasmas and ureaplasmas infection and male infertility: a systematic review and meta-analysis

C. Huang¹, H. Zhu¹, L. Fan², W. Zhu²

¹Central South University, Institute of Reproduction and Stem Cell Engineering, Changsha Hunan, China

²Reproductive and Genetic Hospital of CITIC-Xiangya, Sperm Bank, Changsha Hunan, China

Study question: The goals of this study were to evaluate the association between genital Ureaplasma (*U. urealyticum* and *U. parvum*), Mycoplasmas (*M. genitalium* and *M. hominis*) and risk of male infertility, and to compare the prevalence of genital Ureaplasma and Mycoplasmas infection in China relative to the world average

Summary answer: Our analysis supports that *U. Urealyticum* and *M. Hominis*, but not *U. Parvum* and *M. genitalium* is an etiological agent in male infertility

What is known already: The correlation between Mycoplasmas, Ureaplasmas infection and male infertility has been studied widely. However, The role that *U. urealyticum* and *M. hominis* infections play in male infertility is controversial. Hitherto, *M. genitalium* and *U. parvum* have seldom been investigated in infertile men.

Study design, size, duration: There were 20 studies in this research between January 2000 and December 2014. Nineteen studies with 3975 case and 2249 controls were concerning *U. urealyticum* infection and 9 studies with 2410 cases and 1223 controls were about *M. hominis* infection. Other two infection (*U. parvum* and *M. genitalium*) were studied in 5 and 3 studies, respectively.

Participants/materials, setting, methods: The major criteria were as follows: (a) case-control studies about the associations of genital Ureaplasma or Mycoplasmas with male infertility; (b) the patient group was men who were diagnosed with infertile. The major exclusion criteria were as follows: (a) duplicate data; (b) abstract, comment, review and editorial.

Main results and the role of chance: This meta-analysis indicated that the *U. parvum* and *M. genitalium* might be not associated with the risk of male infertility. However, Ureaplasma urealyticum and Mycoplasma hominis was significantly associated with increased risk of male infertility [ORs were 3.81 (2.48–5.85) $P < 0.00001$; 1.84 (0.93–3.64) $P = 0.025$]. Compared to the world average, a significantly higher positive rate of Ureaplasma urealyticum was observed in both the infertile and control groups in China. In contrast, a significantly lower positive rate of Mycoplasma hominis was observed in both the infertile and control groups in China.

Limitations, reason for caution: First, the sample size was small, which might potentially influence the combined results. Second, other environmental factors, such as smoking and drinking, were not considered in our meta-analysis due to data deficiency. Third, this meta-analysis was conducted based on case-control study that has risk of recall bias.

Wider implications of the findings: More detailed studies of these four species in China and the world could contribute to a better understanding of the epidemiology and pathogenesis, and facilitate the development of better strategies for treatment and prevention of male infertility.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by Changsha City Science and Technology Project (K1106001-31).

Trial registration number: NA.

Keywords: *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, male infertility

P-073 Short-term hypothermic storage of human spermatozoa in electrolyte free medium (EFM): outcomes of 96 IVF cycles

D. A. Isaev¹, I. V. Kapralova¹, E. V. Zakharaeva¹, T. V. Kartavenko¹,

I. S. Krivokharchenko¹, O. O. Zharskaya¹, V. V. Zaletova²

¹Center for Reproductive Medicine MAMA, Embryology, Moscow, Russia C.I.S.

²Center for Reproductive Medicine MAMA, Medical Department, Moscow, Russia C.I.S.

Study question: To determine whether short-term hypothermic storage of human spermatozoa in EFM is effective in routine practice for IVF/ICSI.

Summary answer: Short-term hypothermic storage of human spermatozoa in EFM for up to 2 weeks is safe and effective, and may be used in IVF programs.

What is known already: Storage of human sperm at +4°C in EFM composed of glucose and bovine serum albumin allows sperm preservation for at least 2 weeks (Saito et al., 1996; Kanno et al., 1998). This method was used to generate healthy murine offspring and was shown to be genetically safe (Riel et al., 2007, 2011). Short-term hypothermic storage of human spermatozoa in EFM has not been utilized in clinical practice for infertility treatment with IVF.

Study design, size, duration: This study included 96 couples who underwent IVF treatment between September 2010 and December 2013. Normospermia was the main requirement for participation. After 2-week hypothermic storage in EFM, the spermatozoa were used for fertilization by ICSI.

Participants/materials, setting, methods: Sperm was stored in EFM at +4°C and further processed according to standard protocol. Sperm motility and sperm DNA fragmentation were evaluated before and after hypothermic storage in EFM. Each newborn's physical status was evaluated by questionnaires sent to physicians and parents at conception and delivery.

Main results and the role of chance: After 2-week hypothermic storage in EFM, 56.2 ± 5.0% of spermatozoa regained motility. The sperm DNA fragmentation rate was slightly higher after than before storage (11.2 ± 3.1 vs. 8.5 ± 2.5%, $p = 0.11$), but the difference was not statistically significant. The fertilization rate was 78%, and the clinical pregnancy rate was 34.4% (33 of 96), with 26 pregnancies resulting in the successful delivery of 34 babies. Delivery date and birth length and weight complied on average with standards.

Limitations, reason for caution: The method was effective for ejaculated sperm with normal parameters stored for a period of up to 2 weeks.

Wider implications of the findings: Hypothermic storage of human spermatozoa in EFM is a simple and cost-effective option, if ejaculated sperm cannot be retrieved on the day of ovarian puncture. The method guarantees that the spermatozoa are safely stored for at least 2 weeks, and then regain their motility and viability, with preservation of DNA integrity. Hypothermic storage of human spermatozoa in our IVF/ICSI programs resulted in a high pregnancy rate and high physical status scores in the resulting newborns.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Center for reproductive medicine MAMA, Moscow, Russia C.I.S.

Trial registration number: NA.

Keywords: sperm, hypothermic storage

P-074 Who is the most suitable candidate for varicocele ligation?

– Findings from a cross-sectional survey in a cohort of infertile Caucasian-European patients

A. Serino¹, L. Boeri¹, E. Ventimiglia¹, S. Ippolito¹, A. Pecoraro¹, M. Paciotti¹, P. Capogrosso¹, G. Castagna¹, F. Castiglione¹, A. Russo¹, U. Capitanio¹, F. Cantillo², R. Damiano², A. Salonia¹

¹IRCCS Ospedale San Raffaele, Division of Experimental Oncology/Unit of Urology URI, Milan, Italy

²Magna Graecia University, Research Doctorate Program in Urology, Catanzaro, Italy

Study question: We assessed i) the impact of health-significant comorbidities and hormonal milieu on semen parameters; and, ii) the main predictors of oligospermia in order to identify patients who will not significantly benefit from varicocele ligation.

Summary answer: Current findings demonstrate that almost half of patients presenting for couple's infertility had a clinically-significant varicocele. Our data would suggest not to submit infertile men with high serum levels of FSH to varicocele ligation.

What is known already: According to current EAU guidelines, clinical varicocele should be repaired in infertile patients with oligospermia and an infertility duration of ≥2 years. Indeed, patients' comorbidity profile and hormonal milieu seems to have no importance for therapeutic decision making.

Study design, size, duration: Complete data from 2100 consecutive infertile men were analyzed in a retrospective fashion.

Participants/materials, setting, methods: Comorbidities were scored with the Charlson Comorbidity Index (CCI; categorized 0 vs ≥1). Testicular volume was assessed with a Prader orchidometer. Color-Doppler US was used to detect spermatic vein reflux and to classify the grade of varicocele. Semen analysis values were assessed based on the 2010 WHO reference criteria. Descriptive

statistics detailed the association between varicocele, clinical comorbidities and seminal parameters. Logistic regression models tested the association between clinical predictors and oligospermia. Serum FSH was included in the model as both a continuous and a categorized variable (according to the most informative cut-off: 11.1 mUI/ml).

Main results and the role of chance: Overall, varicocele was found in 982 (46.8%) patients. Patients with varicocele had a mean (SD) age, BMI and Prader of 42.5 (6.7), 25.46 (3.3) kg/m² and 16.48 (5.9), respectively. Patients with varicocele and CCI ≥1 presented a higher prevalence of oligospermia (x2: 10.8; $p < 0.001$); conversely, no differences were found in terms of rates of either asteno- or teratozoospermia. Patients with serum FSH > 11.1 mUI/ml more frequently had a higher BMI ($p = 0.04$), lower testicular volume ($p < 0.001$), lower serum inhibin B ($p < 0.001$) and lower AMH ($p < 0.001$), and testosterone levels ($p < 0.001$). At MVA, FSH was significantly associated with oligospermia as either a continuous (OR: 1.13; $p < 0.001$) or a categorical predictor (OR: 3.8; $p < 0.001$), after accounting for other variables. Likewise, CCI ≥1 (OR: 3.3; $p = 0.001$), duration of infertility (OR:1.1; $p = 0.03$) and testicular volume (OR:0.9; $p < 0.001$) achieved independent predictor status for oligospermia. Conversely, patient age and varicocele were not significantly associated with oligospermia.

Limitations, reason for caution: Cross-sectional, retrospective analyses.

Wider implications of the findings: A general consensus regarding indications for varicocele ligation still lacks. A better selection of patients would allow to identify who will not significantly benefit from surgery.

Study funding/competing interest(s): Funding by hospital/clinic(s) – None.

Trial registration number: NA.

Keywords: varicocele ligation, oligospermia

P-075 Routine determination of sperm DNA fragmentation incorporating the sperm degradation index (SDi) is a useful noninvasive biomarker to identify patients with varicocele in seminograms

J. L. Fernandez¹, J. Gosálvez², C. Lopez-Fernandez², S. D. Johnston³, S. C. Esteves⁴

¹Complejo Hospitalario Universitario A Coruña, Genetics Unit, La Coruña, Spain

²Universidad Autónoma de Madrid, Genetics Unit, Madrid, Spain

³University of Queensland, School of Agriculture and Food Science, Gatton, Australia

⁴Androfert Centro de Reprodução Masculina, Andrology, Sao Paulo, Brazil

Study question: The study attempted to establish the incidence of sperm with degraded nuclei in a large population of semen samples obtained from donors and patients of varying fertility to explore the value of using this parameter as a noninvasive marker of varicocele.

Summary answer: Although not pathognomonic, if a neat ejaculate shows at least 1 in 3 spermatozoa with degraded nucleus, then there is high probability that the individual has varicocele.

What is known already: Sperm with degraded nucleus are a specific subpopulation of spermatozoa identified by means of the Sperm Chromatin Dispersion test (SCDt) that are characterized by massive levels of single and double strand DNA breaks and alteration of nuclear proteins. A high proportion of these sperm with degraded nucleus has been repeatedly observed in varicocele patients but its relative presence has not been systematically analyzed in patients exhibiting different sperm pathologies.

Study design, size, duration: Retrospective international multicenter study consisting of 1528 semen samples obtained from (1) Non-varicocele subjects: including fertile donors, patients with leukocytospermia, cancer or idiopathic infertility, and (2) Varicocele patients exhibiting clinical and subclinical varicocele.

Participants/materials, setting, methods: Sperm with degraded nucleus were identified using the Sperm Chromatin Dispersion test (Halotech DNA, Madrid), protein staining and 2-dimensional neutral and alkaline comets. The proportion of sperm with degraded nuclei in the total population with fragmented DNA was established as the Sperm Degradation index (SDi).

Main results and the role of chance: SDi was significantly higher in individuals diagnosed with varicocele (mean = 0.54; SD = 0.16; Range = 0.227 – 0.952) compared with non-varicocele. ROC curve analysis revealed that a SDi value of greater than 0.32 was able to identify varicocele patients with a sensitivity of 93%, a specificity of 90%, a negative predictive value of 99.5% and an area