Ig G-, but also Ig A- and Ig M-class Ab, and which can be applied to seminal plasma (SP) samples. The clinical relevance during infertility investigation was tested in this prospective study.

Materials and Methods: A total of 173 males from randomly chosen subfertile couples without symptoms of genital tract infections (median duration of infertility 3.5 (range 1-19) years) were enrolled in the study (median age 33 (range 22-52) years). Their female partners were examined at the same time (median age 31 (range 21-43) years). Couples were not selected with regard to infertility factors (primary infertility 89.1%/ secondary infertility 10.9%). Serum of male patients and their partners and same-day seminal plasma samples were screened for anti-chlam. Ab of the Ig G-, Ig A- and Ig M- classes using a commercial recombinant enzyme-linked immunosorbent assay (ELISA) based on chlamydial lipopolysaccachide (LPS) fragment antigens. The outcome of chlamydial serology (with a total of nine samples per couple) was analyzed with regard to multiple parameters of semen quality (evaluated the same day): e.g. results of microscopical sperm analysis, semen cultures, seminal leukocytes (LC) as potential marker of silent infection/inflammation and sperm ability (under standardized in vitro conditions) to penetrate the cervical mucus (CM) barrier as a significant determinant of 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).

Result(s): Anti-chlamydial Ab of the three Ig classes in serum of male patients were significantly interrelated (e.g. Ig M/Ig A Ab $p < 0.01$). Chlam Ig A class Ab in seminal fluid were significantly associated with findings in same-day serum ($p < 0.001$). Evidence for previous chlamydial infection was significantly more frequent in female partners of males with anti-chlam Ab in serum ($p < 0.001$), and also of partners of patients with Chlam Ig A Ab in their seminal plasma ($p < 0.005$). The outcome of chlamydial serology (Chlam Ig G-, Ig A- and IG M-class Ab) in serum, as well as in same-day SP, was not significantly related to results of clinical examination and sperm quality, evaluated with standard semen analysis, seminal leukocytes, and semen cultures. There was no significant relationship of Chlam serology in both partners with the outcome of sperm-CM interaction in vitro and with results of PCT.

Conclusion(s): As part of a comprehensive investigation of male fertility, the outcome of chlamydial serology in serum and same-day seminal plasma was not indicative of semen quality and sperm functional capacity.

P-004 Cryopreservation of single spermatozoa using CryoTop

Y. Endou[1], Y. Fjii[1], H. Motoyama[1]
[1]*Kurashiki Medical Clinic, Medical Science Labolatory, Kurashiki, Japan*

Introduction: Cryopreservation of surgically retrieved spermatozoa from epididymis and testis is valuable component in effective treatment and management of male infertility, reducing the necessity of repeat surgeries. The conventional freezing procedure for these spermatozoa is not appropriate because of their very low numbers and poor in-situ motility. Some authors have reported pregnancies using a few spermatozoa stored in empty oocyte zona pellucida. However, the latter procedure generates many ethical problems and is no longer used. Lack of easily implemented technology to handle remains a major deterrent to single spermatozoa freezing. We tried to cryopreserve single spermatozoa using CryoTop (Kitazato Biopharma, Japan), which consisted of fine polypropylene strip attached to plastic handle and equipped with a cover straw. CryoTop is special container for vitrification of oocytes/embryos.

Materials and Methods: The study also examined two different freezing medium: 0.1M sucrose (Sigma, USA) or 41% (v/v) SpermFreeze (FertiPro, Belguim) in Hepes buffered modified HFF99 (Fuso Pharmaceutical Industries, Japan) with 20% of Serum Substitute Supplement (Irvine, USA). Spermatozoa (n = 100) from 10 infertile men were used to evaluate the efficiency of the method. One microL freezing medium droplets were deposited on the CryoTop strips at room temperature. Individual motile sperm were loaded into it with aid of microscope and micromanipulation equipment. They were cooled in vapor of liquid nitrogen for 2 min and them immersed in it. On thawing, the CryoTops were taken out of liquid nitrogen and instantly placed in 2 microL medium droplets covered by oil at 37?. Each droplet was carefully checked to retrieve sperm by means of an inverted microscope at × 100 magnification. Sperm recovery and motility parameters were assessed. There was no medical fertilization of human oocytes in these sperm studies.

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

F.Q. Quintana[1], Z.L. Zaloa Larreategui[2], I.P. Iratxe Peñalba[1], S.O. Sara Ortega[1], M.M. Monica Martin[1], G.Q. Guillermo Quea[3], J.S. Jose Serna[3]
[1]*IVI Bilbao, Andrology laboratory, Bilbao, Spain*
[2]*IVI Bilbao, IVF laboratory, Bilbao, Spain*
[3]*IVI Zaragoza, Gynecologist, Bilbao, Spain*

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.

Material 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, by masturbation after a minimum of two days of abstinence. Kruger´s strict criteria were applied to evaluate sperm morphology. According to percentage of normal forms, samples were classified into Group A (1-6% normal sperms) and Group B (7-14%). Pregnancy Rate (PR) and Miscarriage Rate (MR) were compared in both groups. *t*-test was applied.

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 morphology seems to show similar results. In the view of these results, there is no clinical usefulness of morphological classification at least to predict PR and MR.

P-006 Oral anti-oxidant use for male partners of couples undergoing fertility treatments

M.G. Showell[1], J. Brown[1], A. Yazdani[2], M.T. Stankiewicz[3], R.J. Hart[4]
[1]*University of Auckland, Obstetrics and Gynaecology, Auckland, New Zealand*
[2]*Clinical Research and Development, Queensland Fertility Group, Woolloongabba, Australia*
[3]*Reproductive Medicine, Flinders Reproductive Medicine, Bedford Park, Australia*
[4]*University of Western Australia, School of Women's and Infants Health King Edward Memorial Hospital and Fertility Specialists of Western Australia, Subiaco, Australia*

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, $I^2 = 0\%$, $p < 0.006$)). 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 6.49, $I^2 = 0$, $p < 0.00001$)). 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, $I^2 = 0\%$, $p < 0.00001$)).

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 = < 0.00001)) in favour of the antioxidant group over the placebo.

Total sperm motility: Antioxidant supplementation in men compared to placebo was associated with an improvement in total sperm motility for the following timeframes:

1. at ≤3 months: pooled OR 9.88 (7.17 to 12.59; I^2 52%, $p < 0.00001$). 348 participants studied in seven trials.
2. at 6 months pooled OR 4.19 (3.81 to 4.56; I^2 89%, $p < 0.00001$). 915 participants studied in seven trials.
3. at ≥9 months: pooled OR 1.38, (0.81 to 1.95; I^2 64%, $p < 0.00001$). 332 participants in three trials.

Sperm concentration antioxidant use compared to placebo was associated with an improvement in sperm concentration within the following time frames:

1. at ≤3 months: There was no beneficial effect determined; pooled OR 2.64, (-0.52 to 5.81; $p = 0.10$), six trials of 290 participants.
2. at 6 months; pooled OR 5.25, (4.43 to 6.08; $p < 0.00001$; I^2 53%), six trials of 825 participants.
3. at ≥9 months; pooled OR 1.61 (0.61 to 2.61; P = 0.002, I^2 0%), three trials of 332 participants.

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-007 Isolation and identification of a protein from human oviductal secretion that interact with human spermatozoa

C. Zumoffen[1], M.J. Munuce[1], A. Caille[1], S. Ghersevich[1]
[1]*School of Biochemical and Pharmaceutical Sciences, Laboratory of Reproductive Studies - Clinical Biochemistry, Rosario, Argentina*

Introduction: After ejaculation, a number of spermatozoa move to the first portion of the oviduct, where they can remain viable during hours or even days in contact with the oviductal secretion and can undergo several metabolic and functional changes to become fertilizing-competent (process known as capacitation). We have found that proteins from conditioned medium (CM) of human oviductal tissue cultures can modulate some sperm functions. It have been suggested that some oviductal proteins interact with human spermatozoa and could modulate sperm function. Thus, the aim of the present study was to isolate and identify proteins from CM with capacity to interact with human spermatozoa.

Materials and Methods: Human oviductal tissue was obtained from premenopausal women (age: 41.0 ± 1.6, n = 20) with no clinical history of infection or cancer disease, scheduled for routine hysterectomies. Native human oviductal fluid (nOF) was recovered by flushing the tubes with DMEM/Ham F12 medium. Explants of tubal tissues were cultured in DMEM/Ham F12 medium at 37 ºC and 5 % CO_2 for 24 h, followed by a further incubation with [^{35}S]Met (30 µCi/ml) during 24 hs, to obtain *de novo* [^{35}S]Met-proteins. After incubation, CM was collected and centrifuged 5 min at 700 × g, to remove debris. The CM were then dialysed, lyophilised and stored at -70ºC until use. Total protein concentration in CM was determined using the Bradford´s assay. Human spermatozoa were obtained from normozoospermic samples of healthy donors (n = 4) after 3 days of sexual abstinence. Motile sperm were recovered by swim-up and were incubated under capacitating conditions in Ham F10 medium supplemented with 3.5 % BSA for 2 h in the absence or the presence of [^{35}S]Met-proteins from CM. At the end of incubations, sperm membrane proteins (SMP) were extracted, analysed by SDS-PAGE (10%) and electrophoretically transferred to nitrocellulose membranes. In addition, a chromatographic affinity column was prepared with motile sperm membrane extracts coupled to Sepharosa 4B, in which CM was seeded. The eluted protein fractions were subjected to SDS-PAGE 10%. The protein bands were analysed by LC-MS/MS. The presence of the identified protein in nOF and CM was examined by Western blot analysis.

Results: A protein band with an estimated molecular weight (MW) of 14 kDa was detected in the autoradiographies of SMP from spermatozoa that were incubated with [^{35}S]Met-labelled oviductal proteins. The SDS-PAGE of eluted protein fractions obtained by affinity chromatography showed the presence of at least five [^{35}S]Met-protein bands by autoradiography, with estimated MW of 127 kDa, 94 kDa, 79 kDa, 17 kDa and 14 kDa, respectively. The 14 kDa protein was identified as human calgranulin B by LC-MS/MS analysis. The presence of calgranulin B in CM and nOF was confirmed by Western blot using specific antibodies.

Conclusion: At least five oviductal proteins that interact with sperm membrane were detected (127 kDa, 94 kDa, 79 kDa, 17 kDa and 14 kDa). In the protein extract from sperm incubated in the presence of CM a protein of approximately 14 kDa was also present. This protein was identified as human calgranulin B and it was detected both in CM and nOF by Western blot. These results support the hypothesis that some oviductal proteins could modulate the sperm function through direct binding on sperm and could have an active role in the fertilization process.

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P-008 Variations in folate pathway genes are associated with male infertility

A.M. Lendinez[1], B. Perez-Nevot[1], A.R. Palomares[2], A. Serrano Garballo[1], A. Rodriguez[3], A. Reche[4], A. Mayor-Olea[5], M. Ruiz-Galdón[5], A. Reyes-Engel[5]
[1]*Hospital Clínico Universitario Virgen de la Victoria, Análisis Clínicos, Malaga, Spain*
[2]*Instituto de Fertilidad Clínica Rincón, Bioquimica y Biología Molecular, Malaga, Spain*
[3]*Hospital Clínico Universitario Virgen de la Victoria, Ginecología y Obstetricia, Malaga, Spain*
[4]*Hospital Materno Infantil. Carlos Haya, Ginecologia y Obstetricia, Malaga, Spain*
[5]*Facultad de Medicina. Universidad de Málaga, Bioquimica y Biología Molecular, Malaga, Spain*

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 infertile males.

Materials and Methods: A group of 28 infertile men (classified according to WHO and Kruger criteria) and 122 controls were genotyped for the following