Main results and the role of chance: The phosphoproteomic study shows a decrease in the phosphorylation of four protein targets related to the calcium signaling pathway, after 1 minute of U50488 treatment. Among these potential proteins, CABYR is a Calcium-binding tyrosine phosphorylation-regulated protein, SPANX is a sperm protein associated with the nucleus on X chromosome, PGRMC2 is the Membrane-associated progesterone receptor component 2 and the FAM71B protein, is directly related to the CatSper sperm specific calcium channel. At the same time, CABYR and SPANX, are two testis specific proteins whose expression is limited to the testis and spermatozoa. These results highlights the fact that the U50488 stimulates a novel calcium signalling pathway which differs from those found in somatic cells supporting the idea that GPCR present unique features in their molecular mechanisms. Moreover, this finding could describe the first steps of the signalling pathways activated via the kappa-opioid receptor and which last in an inhibition of the acrosome reaction at one hour, as the functional studies confirm. This outcome is consistent with the idea that the GPCR, participate in the regulation of the acquistion of the fertile capacity in human sperm via cell specific molecular mechanism.

Limitations, reasons for caution: We need further studies to analyze more in deep the molecular mechanisms by which these 4 proteins participate in the inhibition of the acrosome reaction in human spermatozoa.

Wider implications of the findings: A better understanding of the molecular mechanisms that are involved in the physiology of the spermatozoid and differ from the somatic cells could be very helpful to grasp the aetiology of many cases of infertility and to develop new therapeutic targets and strategies.

Trial registration number: CEISH/61/2011.

O-129 Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of sperm DNA fragmentation: systematic review and meta-analysis

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Study question: To assess outcomes of intracytoplasmic sperm injection (ICSI) using testicular compared with ejaculated sperm in men with high sperm DNA fragmentation.

Summary answer: The existing evidence supports the use of testicular sperm in preference over ejaculated sperm for ICSI in men with high sperm DNA fragmentation (SDF).

What is known already: Recent studies have suggested potential benefits of using testicular versus ejaculated sperm for ICSI among couples with repeated ICSI failure. Oxidative-induced SDF during epididymis transit has been postulated to lower ejaculate sperm quality that may negatively impact ICSI outcomes. However, the evidence is not unequivocal, and some studies have found no benefit of testicular versus ejaculated sperm among men with cryptozoospermia. Therefore, we sought to assess the available evidence concerning outcomes of ICSI using testicular versus ejaculated sperm among infertile men with a strong rationale for using testicular sperm, namely, those with confirmed post-testicular sperm DNA fragmentation.

Study design, size, duration: We conducted a systematic search using PubMed, Scielo, and Google Scholar to identify all relevant studies published until December 2016. The search combined terms related to "sperm DNA fragmentation", "sperm DNA damage", "sperm chromatin integrity OR damage", "testicular sperm", "ejaculate", "intracytoplasmic sperm injection", with the filters "human" in any language. For the advanced search, article types selected were: clinical study, comparative study, journal article, meta-analysis, observational study, randomized controlled trial, review, and systematic review.

Participants/materials, setting, methods: Participants were couples undergoing ICSI with the use of ejaculated sperm (Eja-ICSI) or testicular sperm (Testi-ICSI) whose male partners had normo-/oligozoospermia and high SDF. Studies that included men with unexamined SDF were excluded. Subgroup analysis included the comparison among SDF testing methods. The levels of SDF in ejaculated and testicular sperm and live birth rates (LBR) were the primary outcomes. The secondary outcomes were clinical pregnancy rates (CPR), miscarriage rates, and fertilization rates.

Main results and the role of chance: Our electronic search retrieved 112 articles. After screening titles and abstracts, 11 articles were deemed eligible for full-text evaluation. Among these, we excluded four articles with reasons and included seven studies, involving 507 cycles and 3,840 injected oocytes, for qualitative and quantitative analysis. SDF rates were lower in testicular than in ejaculated sperm (Mean difference [MD] -24.58% [95% CI -32.53%,-16.64%, $P < 0.001$). The live birth rates per embryo transfer were higher when testicular sperm were used for ICSI compared with ejaculated sperm (Odds ratio [OR] 2.35 [95% CI 1.40–3.94; $P < 0.001$). Fertilization rates were not different between the two sperm sources, but the Testi-ICSI group had a trend to lower fertilization rates (OR 0.82 [95% CI 0.58–1.16]. Pooled results showed that CPRs were higher when testicular rather than ejaculated sperm was used for ICSI (OR 2.27 [95% CI 1.48–3.49; $P < 0.001$) whereas miscarriage rates were reduced with the former (OR 0.34 [95% CI 0.15–0.77; $P = 0.01$). Overall, heterogeneity was low but for SDF levels between testicular and ejaculated sperm. Subgroup analysis based on SDF method reduced heterogeneity estimates. Sensitivity analyses did not affect the overall effect size of pooled estimates.

Limitations, reasons for caution: Not all risk factors such as participant age, use of medication, and smoking, which might have affected SDF rates and ICSI outcomes were consistently reported. All included studies were observational. Another limitation refers to the quality of included studies, which also varied.

Wider implications of the findings: Pooled results from evaluated studies suggest that testicular sperm is preferred over ejaculated sperm for ICSI among men with high levels of SDF in the semen. The full clinical implications of using Testi-ICSI for men with high SDF deserves further investigation using randomized controlled trials.

Trial registration number: NA.

O-130 Topographic mapping of sperm chromatin fragmentation within the male reproductive tract and associated reproductive outcomes

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Study question: To assess sperm chromatin fragmentation (SCF) of sperm isolated from various levels of the male reproductive tract and how it affects reproductive outcomes.

Summary answer: There is an increase in SCF from the testes to the ejaculate, suggesting progressively increasing oxidative stressors through the male reproductive tract.

What is known already: The integrity of DNA in sperm has important fertility-related implications. During the later stages of spermiogenesis, breakage of a sizable amount of single- or double-stranded DNA occurs to allow tight chromatin compaction. Much of this DNA breakage is repaired, although reactive oxygen species (ROS) within the male reproductive tract can cause additional damage. While seminal anti-bodies can protect against damage by ROS in the ejaculate, DNA may still remain considerably fragmented. The topography mapping of SCF is particularly important in men with high SCF in ejaculates, where retrieving spermatozoa from the epididymis or testis may bypass such a chromatinic insult.

Study design, size, duration: Men with high SCF in their ejaculates underwent urologic evaluation and bilateral surgical sampling from vas deferens, epididymis, and testis. SCF of the ejaculated and surgically retrieved sperm samples was assessed by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Clinical outcomes for men undergoing ICSI treatment was recorded and stratified by the sperm source.

Participants/materials, setting, methods: Ejaculated samples were processed in standard fashion for SCF assessment with TUNEL. Surgical samples were minced and prepared for TUNEL, after which they were cryopreserved; these samples were thawed at a later date for ICSI treatment. SCF was measured by TUNEL on specimens isolated from all surgical sites. A commercially available

kit was used for SCF assessment and at least 500 spermatozoa were counted per site under fluorescent microscopy with an adopted threshold of 15%.

Main results and the role of chance: Of the original 86 patients, 73 were treated by ART with an average SCF of 30.8 ± 18.4% (range 7.7–96.0). In 10 men aspiration of the vas deferens resulted in 16.4 ± 8% SCF (range 5.8–30.0) while in 44 men epididymal sampling yielded 15.8 ± 6.8% SCF (range 5.3–34.8) and in 83 the SCF on testicular spermatozoa was 11.3 ± 5.2% (range 2.0–27.0). The SCF progressively decreased as TUNEL was performed proximally from the ejaculate toward the vas deferens ($P = 0.05$), the epididymis ($P = 0.01$), and testis ($P = 0.01$). ART outcome with ICSI utilizing these surgical sources yielded a clinical pregnancy of 29.8%, while with the ejaculated counterpart only 16%. Based on these preliminary findings, a subgroup of patients (n = 19) with SCF of 40.1 ± 18% bypassed the prerequisite cycle with ejaculated spermatozoa. By opting to directly undergo TESE with ICSI, a clinical pregnancy rate of 36.0% per cycle was achieved that translated to 62% per couple treated.

Limitations, reasons for caution: Surgical sampling of the vas deferens, epididymis, and testis in men with high SCF in their ejaculates should only be performed after extensive and individualized counseling. Moreover, such an approach is preliminary and requires further evidence, given that some men may not achieve a pregnancy even with surgically retrieved sperm.

Wider implications of the findings: The topographic mapping of SCF evidenced that oxidative stressors progressively compromise DNA integrity toward the ejaculate. Thus, men with high SCF in their ejaculates who are unable to achieve a pregnancy may benefit from undergoing surgical retrieval of sperm for diagnostic and therapeutic purposes.

Trial registration number: Not applicable.

O-131 ORP: a reliable and reproducible method of evaluating oxidative stress - a multicenter study

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Study question: To investigate the reproducibility and reliability of ORP measurement as an indicator for semen quality across different fertility centers.

Summary answer: ORP is a reproducible and reliable method to differentiate fertile from infertile semen samples.

What is known already: Seminal oxidative stress (OS) is well reported to affect male fertility status. The discrepancy in the measurement of OS has hindered its clinical use as a quality indicator for semen. Few studies measured reactive oxygen species alone while others measured only reductants leading to lack of standardization of results. Oxidation reduction potential (ORP) is a better representative for OS as it provides an overall measure of the activity of both oxidants and reductants. Very recently, ORP assessment by MiOXSYS has been introduced as a measure of OS with high specificity in differentiating fertile from infertile semen samples.

Study design, size, duration: This is a multicenter retrospective study comparing data from semen analysis and ORP measurement between two andrology laboratories in two different demographic areas (USA and Qatar) over a period of 1 year (2015 – 2016).

Participants/materials, setting, methods: The same protocol was followed by both laboratories. Semen analysis was performed according to WHO, Fifth Edition. ORP was measured using MiOXSYS. Each data set contains infertile patients' group and normal fertile donors group. Data was analyzed separately from each laboratory. The data from both laboratories was then combined and analyzed. To compare between two groups a Student's t-test was used. Receiver operator characteristic (ROC) analyses were used to determine cutoff values for sORP.

Main results and the role of chance: The first data set from USA contains 194 patients and 51 fertile donors, while the second data set from Qatar contains 400 patients and 50 fertile donors. In both data sets and in combined data, infertile group had significantly lower sperm concentration, total and progressive motility and normal morphology as well as higher ORP level when compared to fertile men ($P < 0.05$).

When comparing data from both centers, the infertile group showed significant difference between both data sets regarding progressive motility and morphology ($P < 0.001$). Also, the percentage of patients with abnormal semen volume, sperm count, total and progressive motility were significantly different between both data sets ($P < 0.05$). sORP level showed no significant difference between both data sets ($P < 0.08$)

ROC analysis indicated that sORP cut-off value of 1.42 mV/10^6/mL in USA group and in Doha group can accurately differentiate fertile from infertile semen groups. When combining both data together the cut-off value for sORP was again found to be 1.42 mV/10^6/mL.

Limitations, reasons for caution: The retrospective design of the study. However all data was available and both centers followed the same protocol.

Wider implications of the findings: Although other semen parameters showed significant differences between the two centers, sORP remained consistent in both data sets individually or in combined data. This proves its reproducibility and reliability. sORP is a measure of semen quality which adds more weight to semen testing in identifying fertile from infertile semen samples.

Trial registration number: N/A.

O-132 Four-dimensional analysis of sperm flagellar waveform as an extension for CASA

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Study question: Analysis of sperm movement in 4D in order to gather information about trajectories, chiralities, rolling and beating pattern for understanding the journey to the oocyte.

Summary answer: 4D analysis of sperm movement revealed a wide variety of swimming behaviors used as an answer to environmental circumstances on the oocytal track.

What is known already: Most past studies were only able to draw on 2D methods like CASA (computer assisted sperm analysis) to investigate swimming behavior and flagellar waveform of sperm. In general, they used projections onto the XY plane provided by conventional microscopic imaging in combination with stop-motion imaging. Previous results show that sperm with a linear trajectory rotate around their long axis at a time span correlated to beat frequency. A lack of rotating leads to a circular trajectory. Furthermore, flagellar excursions in the XY plane were described.

Study design, size, duration: Murine, bovine and human sperm were examined in 4D with reference to trajectories, chiralities, rolling behavior, beating pattern and flagellar waveform. A comparison was made between free swimming, adherent and circling sperm as well as a comparison between digital holographic microscopy (DHM) and CASA.

Participants/materials, setting, methods: Murine sperm from NMRI mice (Charles River Labs), bovine ejaculated sperm from a Holstein Friesian bull (HB-No 678525, Rinder-Union West eG) and human sperm from 5 healthy donors (collected under approved ethical protocols) were allowed to swim into physiological buffer at 37 °C. They were used for both, CASA and high speed digital holographic microscopy to record flagellar waveforms and sperm swimming paths in 4 dimensions.

Main results and the role of chance: DHM allows 4D tracking of the head of free-swimming sperm. With distinguishable left and right surfaces of the sperm head, we were able to monitor rolling of sperm around their long axis with correlating changes between left-face- and right-face-downmost configurations. DHM also allows 4D tracking of the flagellum. We were able to identify flagellar excursions into the Z plane as large as the excursions into the XY plane that are travelling down periodically the flagellum as sinusoid waves during each beat cycle. These waves correlate with rolling and beat frequency. The chirality of rolling is always alternating between clockwise and counterclockwise for a roll-counter-roll cycle. Without rolling, sperm obtain a circular trajectory with a planar movement revealed by DHM. Up to now, the current method to analyze sperm is CASA. We performed a comparative measurement between CASA and DHM to demonstrate the benefit of high speed holographic imaging as an extension to the 2D measurements.

Limitations, reasons for caution: Experiments were performed in vitro which means that not all in vivo questions could be answered.

Wider implications of the findings: Our findings will change the concept of sperm movement fundamentally: They indicate a chiral memory in form of a hypothetical elastic linkage within the flagellar machinery which stores torque of