Received: 18 July 2019 | Revised: 24 October 2019 | Accepted: 24 October 2019

DOI: 10.1111/andr.12720

REVIEW ARTICLE

ANDROLOGY WILEY

The current status and future of andrology: A consensus report from the Cairo consensus workshop group

Cairo Consensus Workshop Group

Correspondence
Christopher L. R. Barratt, Systems Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD19SY, UK.
Email: c.barratt@dundee.ac.uk

David Mortimer, Biomedical Inc., Caulfeild Village, West Vancouver, BC, Canada.
Email: david@oozoa.com

Funding information
All authors are very grateful to UEARS 2019 who provided travel and accommodation support for all workshop participants.

Abstract

Background: In attempting to formulate potential WHO guidelines for the diagnosis of male infertility, the Evidence Synthesis Group noted a paucity of high-quality data on which to base key recommendations. As a result, a number of authors suggested that key areas of research/evidence gaps should be identified, so that appropriate funding and policy actions could be undertaken to help address key questions.

Objectives: The overall objective of this Consensus workshop was to clarify current knowledge and deficits in clinical laboratory andrology, so that clear paths for future development could be navigated.

Materials and Methods: Following a detailed literature review, each author, prior to the face-to-face meeting, prepared a summary of their topic and submitted a PowerPoint presentation. The topics covered were (a) Diagnostic testing in male fertility and infertility, (b) Male fertility/infertility in the modern world, (c) Clinical management of male infertility, and (d) The overuse of ICSI. At the meeting in Cairo on February 18, 2019, the evidence was presented and discussed and a series of consensus points agreed.

Results: The paper presents a background and summary of the evidence relating to these four topics and addresses key points of significance. Following discussion of the evidence, a total of 36 consensus points were agreed.

Discussion: The Discussion section presents areas where there was further debate and key areas that were highlighted during the day.

Conclusion: The consensus points provide clear statements of evidence gaps and/or potential future research areas/topics. Appropriate funding streams addressing these can be prioritized and consequently, in the short and medium term, answers provided. By using this strategic approach, andrology can make the rapid progress necessary to address key scientific, clinical, and societal challenges that face our discipline now and in the near future.

KEYWORDS

environmental influences, epigenetics, fertility, genetics, ICSI, male reproductive health, semen analysis, sperm DNA

Workshop members listed in Table 1.

1 | BACKGROUND TO THE CONSENSUS DOCUMENT

The Evidence Synthesis Group formulating potential World Health Organization (WHO) guidelines for the diagnosis of male infertility noted a paucity of high-quality data on which to base key recommendations.[1] Remarkably, even with basic questions, there was not sufficient data to formulate 'low', much less 'strong', recommendations. Moreover, fundamental deficiencies were evident regarding long-standing questions. Overall, this was deemed symptomatic of a much broader 'andrological ignorance'.[1,2]

Suffice it to say that there is a fundamental lack of knowledge that substantially obstructs research, diagnosis, and patient management. Yet, paradoxically male infertility is a part of a dynamic and growing health industry within which ART is a highly innovative, billion-dollar enterprise. Reproductive medicine rapidly attracts the attention of the general public and, as such, the perception is that significant progress has been made. This is an illusion as, for example, numerous basic, clinical, and scientific questions in andrology have remained unanswered—some for over 50 years.[2,4]

Following the first birth of an ICSI-conceived child in 1992, use of ICSI worldwide has increased dramatically and the treatment is increasingly used even when no male problem is present.[5,6] But ICSI treatment is paradoxical: ICSI does not treat male fertility as the man's fertility status remains unchanged (only the gametes are manipulated), and the woman largely carries the treatment burden for male infertility. Furthermore, and ironically, many authors argue that the success of ICSI has focused research onto the female (to optimize the ART process) and thus diverted attention away from identifying the causes and thus rational diagnosis and treatment of male infertility.[7]

Recently, we detailed how this parlous situation was reached and suggested how we might move forward in a structured way to improve our understanding and management of male reproductive health issues.[2] One component of this was to suggest an analysis of the underlying deficits in our knowledge of clinical laboratory andrology and to identify strategies whereby medical and scientific research might seek to fill these gaps. In so doing, we can start to build a sound foundation for the future of andrology as a biomedical specialty: a future in which the male partner in infertile couples is seen as a patient and not merely a source of spermatozoa, in which there are effective first-line treatments for male factor infertility and in which political leaders understand how poor product regulation and neglect and mismanagement of our environment are impacting human fertility, and health in general.

The overall aim of this Consensus workshop (see Table 1 for details of participants) was to clarify the current deficits in knowledge so that clear paths for future development could be navigated. Following presentation of the evidence a total of 36 consensus points are presented.

2 | WORKSHOP SESSION 1—DIAGNOSTIC TESTING IN MALE FERTILITY/INFERTILITY

2.1 | Semen analysis, standardization, and reference ranges

2.1.1 | Origins of standardization in semen analysis

Although the semen sample examined in the laboratory differs significantly to the situation in vivo, it is the standard approach to assessment of male infertility. The first protocol for analysis of human semen that could be considered as suitable for clinical laboratory testing came from Macomber & Sanders.[8] These investigators diluted a known volume of semen with a known volume of bicarbonate/formalin solution to 'dissolve' seminal mucus and formalin to 'stop the activity' of the spermatozoa. After mixing, an aliquot was placed in a blood counting chamber (hemocytometer) allowing calculation of the sperm concentration. They commented on sources of error in the technique and how to proceed when the sperm numbers were either very high or very low and concluded that 'the spermatozoa count has proved a useful guide to fertility'.

In the early 1950s, a series of investigations on human semen quality were published that can arguably be credited with heralding modern andrology.[9] Throughout these investigations, close attention was paid to minimizing confounding variables. For example, the method for counting spermatozoa (hemocytometer) was standardized and a single, gold standard observer (the principal investigator) examined initial motility and counted all specimens. Semen samples from 1000 men of known fertility and 1000 men from infertile marriages were evaluated, and purportedly, it was the results from this report that served as the basis for the reference ranges in the first WHO supported Laboratory Manual for the Examination of Human Semen.[10] Since 1980, the WHO has published four revised editions of the manual containing standardized methods for examining and testing semen (WHO 1987, 1992, 1999, 2010) in an attempt to align the discipline more closely with other standardized clinical laboratory practices.

2.1.2 | WHO (2010) reference values and their impact on clinical and laboratory practice

Strict adherence, by multiple laboratories, to standardized methods for semen testing and evaluation makes data more easily comparable and analysis more statistically powerful, such that ranges of 'reference' values can be derived. The 2010 WHO manual was the first to contain reference values derived from a large set of data.[12] However, a limitation was their derivation from a limited demographic (Europe, Australia, and United States) and, in addition, there was variation in methods used for assessing critical semen parameters.

Ideally, information provided by the WHO should facilitate meaningful interpretation of semen analysis data and conclusions about fecundity both for populations and individual patients. In the

TABLE 1 Participants in the consensus meeting

Participant	Role(s)	Affiliation	ORCID ID
Christopher Barratt	Co-Convenor, presenter, contributor, participant, writer	Systems Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD19SY, UK	0000-0003-0062-9979
David Mortimer	Co-Convenor, presenter, contributor, participant	Oozoa Biomedical Inc, Caulfeild Village, West Vancouver, BC, Canada	0000-0002-0638-2893
Medhat Amer	Presenter, contributor, participant	Department of Andrology, Cairo University, Cairo, Egypt	0000-0001-9129-2333
Elisabetta Baldi	Presenter, contributor, participant	Department Experimental and Clinical Medicine, University of Florence, Florence, Italy.	0000-0003-1808-3097
Christopher De Jonge	Presenter, contributor, participant	University of Minnesota Medical Center, Department of Urology and University of Minnesota Diagnostic Andrology Laboratory, Minneapolis, MN 55 454	0000-0002-4083-5833
Mohamed Fawzy	Local organizer, contributor, participant	Ibnsina and Banon IVF Centers, Sohag and Assiur, Egypt	0000-0001-8756-3612
Niels Jørgensen	Presenter, contributor, participant	University Department of Growth and Reproduction, Rigshospitalet, GR-5064, Blegdamsvej 9, DK-2100 Copenhagen, Denmark	0000-0003-4827-0838
Csilla Krausz	Presenter, contributor, participant	Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy.	0000-0001-6748-8918
Ali Mahran	Presenter, contributor, participant	Dermatology and Andrology Department, Assiut University Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt	0000-0001-7870-4110
Sarah Martins da Silva	Presenter, contributor, participant	Systems Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD19SY, UK	0000-0003-2579-4866
Sharon Mortimer	Contributor, participant	Oozoa Biomedical Inc, Caulfeild Village, West Vancouver, BC, Canada	0000-0002-3498-5667
Allan Pacey	Presenter, contributor, participant	Department of Oncology & Metabolism, University of Sheffield, Sheffield, UK	0000-0002-4387-8871
Stephen Publicover	Presenter, contributor, participant, writer	School of Biosciences, University of Birmingham, B15 2TT, UK	0000-0002-2911-5288

2010 WHO manual, the reference ranges were based on results from relatively few fertile men (428-1941) whose partners had a time to pregnancy of 12 months or less.[12] When reference ranges are calculated in clinical chemistry, it is common to include 95% of obtained data and, usually, the 2.5th and 97.5th centiles form the lower and upper reference limits. The rationale is that both too high and too low values may be considered harmful. However, as high values for semen parameters usually are not considered to impair fertility, the WHO guidelines use a one-tailed approach, basing the reference levels on the 5.0th centiles.[13] WHO emphasizes that 'Men whose semen characteristics fall below the lower limits given are not necessarily infertile'. However, it cannot be concluded that a man will have a normal fertility chance if his semen parameters are above the reference ranges. Several studies have shown that fertilizing ability diminishes if the sperm concentration is below 40-50 million/ml, total sperm count below 140-150 million, the percentage of motile spermatozoa below 70%, and percentage of morphologically normal spermatozoa below 9%-12%.[14-16] These values, which considerably exceed the WHO reference levels (15 million/ml, 39 million, 40%, and 4%, respectively), indicate levels at which ability to conceive may become impaired. It is probable that, in the future, subcategories will need to be introduced to take account of this complexity. Additionally, semen quality must be considered as a continuum and models to predict fertility chances, rather than specific reference levels, must to be developed. Such models should also take into account the potential interaction between the different semen parameters. It is obvious that data obtained from fertile men are relevant to guide us to understand the impact of semen quality. However, adding information from well-defined populations of men with fertility problems may also be relevant to calculation of risk estimates for prolonged waiting time to pregnancies as would, for example, accounting for factors such as female factor and variation between ejaculates.[17]

Unfortunately, the WHO[13] reference values are often wrongly equated with indicating fertility if a sample exceeds all lower reference limits and infertile if it falls below the reference for one or more parameters. A consequence of using reference values as cut-offs is that, as new data emerge and reference values are refined, semen samples may change classification.[18,19] Importantly, it is critical not to use one single semen parameter as a marker of male reproductive potential. Guzick et al[15] formulated three categories: subfertile, fertile, and 'indeterminate'. Odds ratios for infertility climbed dramatically from the combination of all 'fertile' combined sperm parameters (OR 1.0) to all subfertile sperm parameters (OR ~ 16). Sperm parameters in mixed combination had

escalating odds ratios for infertility. The take home message was that significantly greater diagnostic ability comes when the sperm parameter assessments are combined rather than used individually.[1]

2.1.3 | Compliance and quality

To determine whether semen assessments are robust throughout the world requires that there are standardized methods used by clinical and research laboratories. Such standardized methods certainly exist but a wealth of data shows that semen assessments, for a variety of reasons such as lack of adherence to methods, can have poor reproducibility.[1,20-22] As a result, a patient categorized as subfertile in one laboratory has reasonable likelihood of being categorized as fertile in another laboratory and vice versa.

One potential way to correct for lack of standardization is by offering specialized training courses,[24,25] Statistical comparison of participant pre-training to post-training results clearly demonstrates a significant improvement. However, training must be supported by routine external quality assessment (EQA). EQA not only provides a mechanism for assessing testing proficiency of personnel but also compliance with standardized methods for testing. For example, a survey was conducted of andrology workshop-trained and workshop-certified staff and of non-certified laboratory staff working in Polish laboratories to assess compliance with WHO (2010) standards.[26] Regardless of certified training or not, there was an appreciable lack of compliance with, and adherence to, WHO standards. In Italy, an analogous study was conducted in which technologists participated in three trials of EQA assessment. The results from this study showed a high degree of variation in methodology and proficiency, and this in spite of the fact that a training session was provided between trials 2 and 3.[27]

Clearly, a greater unified effort toward continuous training and compliance to improve the standard and consistency of semen analysis is required. The beneficial effects of such compliance and adherence to standards are illustrated by the data of Punjabi who evaluated 15 years of results (1997-2012) from a voluntary EQA program in Belgium.[28] Results showed that adoption by laboratories of the Neubauer hemocytometer as the standard sperm counting chamber resulted in a decrease in CV compared with other chambers used. This study demonstrates that long-term 'buy-in' by administration and laboratory staff to published professional standards leads to success.

2.2 | The current and future potential of sperm functional testing

To fertilize, a spermatozoon must penetrate cervical mucus, pass through the uterus, enter the oviduct, and ascend to the site of fertilization (which probably involves binding to and active escape from the lining cells), locate the oocyte, undergo acrosome reaction, penetrate the cumulus and zona, and fuse with the oocyte, forming the sperm aster and achieving syngamy.[30] These different aspects of the spermatozoon's activity involve a wide variety of functional attributes, impairment of any of which might underlie a failure to fertilize. Tests of sperm attributes/fertilizing ability therefore address a wide range of functional characteristics, for example, motility/hyperactivation assessment, mucus penetration, zona pellucida interaction, acrosome reaction, and oocyte penetration. In addition, biochemical assays and marker assays such as localization of membrane constituents (see below) can be used to assess characteristics believed to be correlated with sperm function. Such tests will address one or more of these key functional attributes and aim to identify samples where a particular attribute (or attributes) are impaired. Since the etiology of male infertility/subfertility can vary (both the functional defect(s) and the causative lesion(s)), such tests could detect subfertility related to a group of lesions with a common functional effect, but might nevertheless detect only a small subset of potential fertility-compromising defects.

It is remarkable that little progress has been made on true sperm function tests since the 1980s/1990s.[31,32] Some, for example, acrosome reaction (AR) and zona binding are reported to have potential clinical value but the literature is not consistent (eg, questioning of both sensitivity and specificity in a recent meta-analysis.[33] Use of these tests often requires access to human biological material (zonae, cervical mucus) and involves complex manipulation requiring high levels of operator training. Moreover, biochemical assays often require technologies that are relatively expensive (eg, flow cytometry, fluorescence plate reader) and/or low throughput. Computer-aided sperm analysis (CASA) is potentially valuable but is also expensive to set up, requires training and is yet to be fully adopted in andrology laboratories.[34] Improvements in technology and development of standardized applications might improve this. To date, CASA has been used primarily for descriptive analysis but the development of functional assays (such as assessment of hyperactivation in capacitating or agonist-stimulated cells) should be an aim.[34,35] Despite this overall lack of progress, there are a number of areas where recent advances suggest that progress in the development and application of new sperm functional tests could occur. These include proteomics of spermatozoa and the seminal plasma,[36] metabolomics and mitochondrial metabolism,[37-39] novel markers of fertilizing ability, for example, the Cap test[40] and the considerable progress being made in understanding of sperm physiology and cell signaling, particularly the expression and functions of spermatozoon ion channels. For illustrative purposes, some examples are briefly considered below.

Mitochondrial status of live spermatozoa can be assessed using fluorescent dyes such as JC-1, either in single cells (by microscopy) or in populations (flow cytometry[41]). Measurements made with JC-1 and other dyes have shown that mitochondrial membrane potential is correlated with markers of semen quality such as motility, viability, count, and morphology[42,43] but diagnostic/predictive value for fertilizing potential has not been established.

The Cap-score assay is based up the relationship between localization of the ganglioside monosialotetrahexosylganglioside (GM1) and capacitation status. Spermatozoa which can undergo acrosome reaction are drawn from a subpopulation that display a 'capacitated' GM-1 pattern where the ganglioside is localized apically.[44]

The staining procedure is carried out on fixed cells so samples can be sent to a central laboratory allowing standardization. In a recent study, where men likely to have trouble conceiving were identified using their Cap-score, the absolute pregnancy rate was almost three times higher in these men considered likely to conceive according to the previously defined Cap-Score reference ranges.[40] However, the data are still preliminary and more clinical trials are required.

Krausz et al[45,46] first showed that the ability of progesterone to induce an immediate Ca^{2+}-influx in human spermatozoa is correlated with fertilization rate at IVF. This action of progesterone is now known to reflect activation of CatSper, the primary Ca^{2+} channel of spermatozoa. Recent work has confirmed this finding and shown similar correlations with other agonists[47,48] but no attempt has yet been made to identify a predictive value (level of response below which fertilization failure is predicted). Men with no detectable $[Ca^{2+}]_i$ response to progesterone (indicating no functioning CatSper) appear to be unable to fertilize naturally or by IVF and thus require ICSI, but extensive screening suggests that this condition is very rare, probably being less than 1% of IVF patients.[45-47,49] A more common defect in spermatozoon ion channel function might be in regulation of plasma membrane potential (V_m). Brown and colleagues, using patch clamping to measure K^+ currents and estimate V_m, found that in > 10% of samples from IVF patients the membrane potential was abnormally depolarized and that a highly depolarized membrane potential ($V_m \geq 0$ mV) may be associated with low fertilization rate.[50]

These and other new assays are potentially of considerable value, though in almost all cases the clinical value is yet to be assessed in large groups of patients. With regard to use in the clinic, the most useful tests will probably be prediction of IUI/IVF/ICSI failure although these are clearly different biological endpoints.[51,52] Two areas of rapid progress provide grounds for guarded optimism: (a) real progress in our understanding of sperm physiology (and consequent identification of new parameters for assay) and (b) improvement of high-throughput assay techniques, including simultaneous flow cytometry/fluorescent imaging. Development of multiplex tests for several sperm attributes, based on flow cytometry or plate reader methods (eg, simultaneous detection of Cap-1 pattern, AR, CatSper expression, mitochondrial V_m), may enable more reliable assessment of sperm functional competence and probability of fertilization failure, facilitating selection of the most appropriate treatment pathway.

2.3 | Sperm DNA assessment

As sperm DNA fragmentation (sDF) is only partially related to semen quality, it could be an important addition to the sperm function testing armamentarium.[53,54] However, there is currently debate regarding its routine use as part of the work-up in cases of male and couple infertility.[55] The most important issue emerging from this debate is the presence of several possible assays, many of them not standardized. These assays vary greatly both in the method and type of damage they are detecting. The type of damage assessed by the different techniques is critical. Indeed, there is evidence that the indirect methods (Sperm Chromatin Structure Assay [SCSA] and sperm chromatin dispersion test [SCD; also known as Halosperm test] detect the susceptibility of chromatin to undergo DNA damage and fragmentation, whereas more direct methods (TUNEL [terminal deoxynucleotidyl transferase dUTP nick end labeling] and Comet [single cell gel electrophoresis assay] actually assess the presence of breaks within the DNA.

As the most important damage is that which affects reproductive outcomes, it is important to clearly define the relationship between the test used and the clinical outcome. This point has been addressed in recent meta-analyses[56-61] where studies were grouped according to the different methods of assessment. When the miscarriage rate was considered as the endpoint, both after ARTs and natural pregnancies, the TUNEL method appeared to be the most useful, followed by SCSA.[59] A more recent meta-analysis, considering couples with repeated pregnancy loss (RPL) following natural conception, revealed a similar, significant association both when TUNEL and SCD were used to evaluate sDF.[61] McQueen et al[57] who also presented a systematic review and meta-analysis of RPL concluded that TUNEL was the most appropriate sDF method. The meta-analysis by Osman, where the endpoint was live birth rate after ARTs, suggested that TUNEL was the most clinically useful technique followed by Comet.[58] Cissen et al[56] who considered clinical pregnancy after IVF and ICSI as the endpoint, showed only TUNEL and Comet to be of 'fair discriminatory capacity', whereas SCSA and SCD tests showed a 'poor predictive capacity'. Finally, Simon et al[60] reported that TUNEL, Comet, and SCD were predictive of clinical pregnancy after IVF and ICSI, with SCD being the least significant. Although the studies included in these meta-analyses differ considerably, and the female factor is often neglected, they report evidence that SCSA and SCD have limited clinical value. Overall, these studies confirm that the different techniques detect different types of damage.

The above conclusion is also supported by studies investigating the effect of sperm selection techniques on DNA integrity. For example, density gradient centrifugation (DGC) may provoke an increase of DNA damage in highly motile selected spermatozoa.[62-64] However, in contrast to assessment using TUNEL, such an increase in DNA damage does not appear to be detected by SCSA.[62] Further studies will be necessary to understand whether Comet and SCD are also able to detect DNA damage after DGC. The scenario appears different in studies on natural conception[65] and intrauterine insemination (IUI),[66] where SCSA results were found to be a reasonable predictor of pregnancy. However, overall, there is an urgent need for more primary data in this arena as meta-analyses are only as strong as the original data available.

Another key challenge is that several different sDF threshold values have been proposed for discriminating between pathological and normal conditions. This inconsistency further contributes to the current confusion. At present, the only possibility is for each laboratory using sDF to set its own cut-off value, suitable for use with the established method, for distinguishing between fertile and subfertile or infertile men. It is anticipated that, once further high-quality clinical data

are available, consensus guidelines can be developed to identify the gold standard method(s) to evaluate sDF for couple infertility work-up.

Emerging data in the literature suggest that several conditions are associated with high SDF levels including varicocoele, unexplained infertility, recurrent IVF or ICSI failure, advanced age, diabetes, and presence of inflammatory signs of the lower genital tract and cancer.[67,68] Patients with these conditions might benefit from evaluation of SDF as diagnostic tool but, with the possible exception of varicocelectomy, effective treatments for diagnosed SDF remain to be determined.

In conclusion, we are still some way from understanding how best to introduce the assessment of SDF into the male infertility work-up. At least three important areas remain: (a) establishing the gold standard technique(s) for each reproductive outcome; (b) finding effective pharmacological treatments to decrease clinically relevant sperm DNA damage in vivo; and (c) establishing correct strategies to prepare spermatozoa for ARTs to avoid iatrogenic damage. Suffice it to say, there is a need for large-scale, robust, controlled trials on the value of sDF testing.

3 | WORKSHOP SESSION 2: MALE FERTILITY/INFERTILITY IN THE MODERN WORLD

The study by Carlsen suggesting that human sperm counts were in the process of significant decline,[69] stimulated great interest while precipitating a lively and ongoing debate about the nature of the data analyzed and whether the interpretation was sound. Data analyzed in subsequent studies appear to confirm that this downward trend is real, at least in men of western countries ([70]; see below), and much of the debate is now moving on to questions of underlying causes and mechanism(s).

3.1 | Is male fertility really declining?

3.1.1 | Temporal trends in semen quality

Meta-analyses show a negative time trend in semen quality

Although a temporal trend in semen quality was discussed briefly in the 1970s,[71,72] the current ongoing debate was started in 1992 by an analysis of semen quality data from 61 studies published between 1938 and 1990.[69] The authors concluded that there was 'Evidence for decreasing quality of semen during past 50 years'. A re-analysis by Swan et al, which included data from a further 47 studies, corroborated the finding.[73] In 2017, the most comprehensive meta-analysis to date was published,[70] incorporating 244 estimates of sperm concentration and total sperm counts, based on 185 studies (including studies on general [unselected] populations and on selected fertile men of 42 935 men who provided semen samples between 1973 and 2011. Criteria for inclusion of studies covered semen collection methods, assessment methods, and statistical analysis. Data analysis took account of age, ejaculation abstinence period, and number of samples from each man. Trends did not differ among studies from Europe/Australia and America so these areas were combined into one group of Western men. The observed trends were dependent on the population group, the largest decline in semen quality being among the Western unselected men where there was a 1.4%/year decrease in sperm concentration (−1.38 million/ml/year; confidence interval −2.02 to −0.74 and 1.6%/year decrease in total sperm count (5.33 million/year; confidence interval −7.56 to −3.11. A less pronounced decrease was detected among the Western fertile men (sperm concentration −0.68 million/ml; confidence interval −1.31 to −0.05 and total sperm count (2.12 million/ml; confidence interval −4.31 to 0.07. When the analysis was restricted to data collected after 1995, the slopes were slightly steeper than for the entire period from 1973, arguing against an attenuation of the adverse trends. No conclusions could be reached about men from Non-Western countries primarily because few studies conducted in these areas have been carried out/reported.

Year of birth may be an important factor

Following the Carlsen publication in 1992, other research groups started to evaluate or re-evaluate data they had previously collected. Examination of 1351 fertile semen donor candidates from Paris during a 20-year period showed a decrease in mean sperm concentration from 89 million/ml in 1973 to 60 million/ml in 1992.[74] After adjustment for age and abstinence period, each successive calendar year of birth was associated with a 2.6% decrease in mean sperm concentration pointing to a birth cohort effect stronger than the effect of the year of examination (−2.1%). Furthermore, sperm motility and morphology were negatively associated with calendar year and year of birth, whereas semen volume was not. This study also showed the importance of accounting for confounders, since greater age was associated with a decreased sperm concentration, percentage of motile spermatozoa, and percentage of morphologically normal spermatozoa, and ejaculation abstinence period was positively associated with sperm concentration and negatively with sperm motility. A subsequent Scottish study, investigating semen donor candidates and not restricted to men with proven fertility, similarly showed deterioration of semen quality as a consequence of later year of birth.[75] A study published in 2012 confirmed the decrease in a population of French fertile donors over a 34-year calendar period, total sperm counts had decreased from 443 to 300 million, without any concomitant change in semen volume.[76] Studies of university students from the southern part of Spain, born between 1974 and 1993, have also shown a decrease in both sperm concentration and total sperm count according to year of birth.[77]

In contrast to the studies described above, no trends among Swedish or Danish men from the general populations have been detected during the last 20 years. The Swedish study compared results from men investigated in 2000-2001 and in 2008-2010 and did not detect any difference between these two time points.[78] Similarly, no

changes were detected among Danish men in the period between 1996 and 2016.[79] However, in a comparison with a historical Danish cohort examined in 1940-1943, where sperm concentration was assessed by the same type of haemocytometer as used in the studies 1996 to 2016, it was shown that recent sperm counts were considerably lower. Sperm concentration had decreased from a median above 60–45 million/ml and total sperm count from more than 300 to 143 million.[80] Vierula et al[81] reported that sperm count of Finnish men was high and unchanged. However, subsequent prospective cohort studies of young men from the general population from south-western Finland detected a decreasing sperm count among men born in the period 1979-1987.[82] Due to the relatively short duration of the investigation and the age of the men, it was not possible to determine whether these changes were best described according to year of birth or year of investigation (1996-2006). In a further investigation of new cohorts of Finnish men, a continued decrease has recently been reported.[83]

As stated above, the meta-analysis by[70] also reported findings for other populations in addition to Western men but did not find any significant declines for studies from South America, Asia, and Africa. This lack of significant findings from non-Western countries may partly be accounted for by limited statistical power and the absence of studies in unselected men in these non-Western countries prior to 1985.[70] The main obstacle to using many historical publications is that many clinical studies on semen quality have dealt with selected groups of men like volunteers enrolled after advertisement, candidates for vasectomy or infertility patients, which hampers interpretation. Since publication of the Carlsen paper in 1992, there have numerous publications based on such populations reporting a downward trend in semen quality and it is beyond the scope of this paper to summarize all. Following the Levine publication in 2017, a meta-analysis of African data from 2017 concluded that there is 'Evidence for decreasing sperm count in African population from 1965 to 2015' based on mixed populations from infertility clinics, laboratory studies, and cohort studies.[84] This conclusion may be true but due to the potential selection biases, the overall pattern in African men still remains unclear. A study of Indian fertile men detected a small downward trend in sperm concentration during a 37-year period and a steeper decline among infertile men. The selection bias for the infertile men was not described, and the methods for assessment of semen parameters were not discussed.[85] Studies from China investigating semen donor candidates where selection biases may be similar over the years have recently been published and described a downward trend for sperm concentrations and motility.[86-88] A minor disadvantage is that assessments were done using Makler chambers which may lead to a lower precision in determination of semen variable,[89] but it provides some evidence that there may also have been a recent change in semen quality among men from various regions in China.

Conclusions about semen quality based on retrospective data

Although the retrospective approaches as described above are not ideal, this should not prevent us from trying to learn about the past. The existing analysis of historical data is the best that can be done, and the results clearly point to adverse temporal trends in semen quality and thereby in a broader sense in male reproductive health, at least among Western men. Interestingly, the association between year of birth and change in semen quality suggests that we should look for reasons among pre-natal events affecting testicular development.[90] This does not exclude the potential importance of post-natal events.[91,92] It is interesting to note that in most Western countries, there have also been increases in incidence of testis cancer concomitantly with the decrease in semen quality.[93] These increases also seem to be more strongly related to the year of birth than the actual calendar year the cancer appears, with a highest incidence among Danish and Norwegian men who apparently also have the lowest sperm counts.[94] Imperfect as they are, these retrospective studies are strong enough to warn us about a current problem and inspire us to establish well-designed studies that can be used not only for longitudinal future measures but also to describe the current situation and potential reasons for declining semen quality.

3.1.2 | Studies designed to detect between-country differences, current situation, and future trends in semen quality

Inspired by these temporal trends in semen quality, cross-sectional, standardized, and coordinated studies have been designed and undertaken to investigate semen quality of men from the general population (unselected regarding fertility status) in primarily western Europe, the United States, and Japan.[94-99] Similarly, standardized and coordinated studies of fertile men have also been undertaken.[100-102] When successfully carried out, such studies will contribute more conclusive information about the existence of differences in semen quality between countries than can be achieved by additional analyses of previously collected data. Furthermore, besides being able to describe the current situation, these data will serve as reference points for future follow-up studies to detect temporal changes and might also provide indications for the cause(s) of declining semen quality.

Selection bias is important to consider when studies are designed

A description of semen quality in a population must rely on results from examinations of a sample of men because it is not possible to examine the entire population. Therefore, since the aim must be to have a representative sample, an important question to consider is whether selection bias might influence outcome.[103] Clearly, selection bias cannot completely be avoided and it is therefore essential that basic variables describing fertility status, health status, lifestyle factors, and educational status are included and analyzed in cross-sectional semen quality studies.[104]

Concern about fecundity of men from general populations

Standardized studies of men from the general populations of Western countries (see above) have shown statistically significant

differences in average semen quality levels between men from different countries. From a biological point of view, these between-country differences are less pronounced than the within-country differences between men from the general population and men of proven fertility. When these studies are interpreted according to the association between semen quality and likelihood to obtain pregnancy,[14-16] rather than the artificial binary reference levels provided by the WHO (WHO 2010, see above discussion), they might indicate that some men from general populations have semen quality that raises concern for their fecundity.[93,105,106] However, these predictions remain to be rigorously tested.

3.2 | Semen quality is not only a marker of fecundity but also health

Recently, it was indicated that impaired semen quality/male infertility is associated with shorter life expectancy and increased long-term morbidity.[107-110] The mechanisms underlying this association are not clearly understood, but reduced semen quality seems to be a marker that is linked to mortality in a dose-dependent manner and also to morbidity. For example, in a Danish population of men from infertile couples, men with a sperm concentration of 195-200 million/ml were, on average, hospitalized for the first time 7 years later than men with a sperm concentration of 0-5 million/ml.[110] Impaired semen quality has been linked to a higher risk of testicular cancer in the years following infertility evaluation, both among Danish and US men,[111] and some studies have suggested a link with development of prostate cancer, although this has not been confirmed by all studies.[68,112,113] Other malignancies such as melanoma, bladder, thyroid, and hematological cancers have also been more frequently observed among infertile men.[67,68] Significant associations between diabetes mellitus, metabolic disorders, and male infertility were found, and infertile men seem to have a higher risk of developing cardiovascular diseases.[107,114] However, incidence of these diseases is not sufficient to explain the observed association between semen quality and mortality.[109] Although it is still not understood which diseases are associated with impaired semen quality, it seems that fertility and especially impaired semen quality might represent a universal biomarker of later health and survival.

Infertility may hence serve as a marker of serious conditions later in life, but this association is in most cases not understood. It has been suggested that adverse lifestyle or environmental exposure during early fetal life, combined with a genetic or epigenetic predisposition, may lead to impairment of reproductive function and endocrine imbalance, which in turn may lead to cancer, osteoporosis, metabolic, and cardiovascular disease. However, the association between fertility and health later in life may also be confounded by current health and lifestyle factors. For example, obesity and smoking are known not only to hamper fertility, but also the general health and life expectancy are negatively affected.[115,116] However, despite the well-known adverse effects of such factors, they do not explain the overall associations between semen quality and morbidity.[117] Most men presenting with low semen quality have normal serum testosterone (T) levels,[118] and for most, the hormonal profile does not indicate immediate need of androgen replacement therapy, but rather suggests that the capacity of the testicular Leydig cells to produce testosterone is decreased. Recently, a compensated reduction in Leydig cell function—assessed as a decreased ratio between testosterone and luteinizing hormone (T/LH)—has been described even in men with semen quality well within the WHO normal reference ranges.[119] Most cases of testosterone deficiency develop through an initial stage of compensated Leydig cell failure,[120] and most likely, Leydig cell function decreases with age.[121] However, it remains to be investigated whether men with subtle Leydig cell failure at young age will develop clinical symptoms of Leydig cell failure at a later stage. Thus, it is currently unresolved to which degree Leydig cell failure contributes as a causative factor to the associations between semen quality and health.

Men with fertility problems represent a relatively large and easily reachable part of the population showing excess mortality and morbidity risk. Therefore, men seeking help for infertility could be a good target for preventive measures aimed to achieve 'healthy aging'. As recognition of this association has only recently emerged, the nature of preventive actions that might be taken to address the increased risk of long-term morbidity and mortality is yet to be established. A major and focused research effort is required to address this issue.

3.3 | Exogenous influences on human male fertility

The reasons for the poor and apparently declining quality of semen from men in many populations remain elusive. A wide range of internal and external environmental variables potentially affects semen quality, both by effects on reproductive function of the mature adult and by effects on development of the male reproductive system *in utero*. A number of male reproductive disorders, including poor spermatogenesis, testicular cancer, hypospadias, and cryptorchidism, have been found to be interrelated—both within individuals and at a population level. These factors, except isolated hypospadias, are associated with reduced fecundity. This has led to the proposal of a testicular dysgenesis syndrome (TDS) originating in fetal life.[90] The potential for male reproductive function seems to be determined during a sensitive period *in utero*. This period has been identified in rodent models showing that normal development and later function of the male reproductive system are highly dependent on adequate androgen production during this sensitive window.[122] It is likely that a similar sensitive period occurs in humans at around gestational weeks 8-14, termed 'the masculinization programming window'. It has been suggested that, in humans, development can be disturbed by genetic defects, epigenetic factors, or adverse exposures, including maternal lifestyle, such as smoking during pregnancy, and exposure to environmental chemicals.[93] Most likely, it is the cumulative effects of various low-dose exposures in our environment, rather than single exposures

that contribute to the appearance of adverse effects in the male reproductive system. Animal studies provide evidence that man-made chemicals can disrupt the hormone dependent pathways responsible for fetal gonadal development, subsequently leading to TDS-like symptoms.[123-125] Causal relationships are inherently difficult to establish in humans. Although the maximum potential for adult semen quality seems to be determined *in utero*, exposure to man-made chemicals in the environment and to other lifestyle-related stresses in the adult also influence semen quality. Additionally, several post-natal factors such as infections, surgical procedures, and medical treatment might cause reduced semen quality in adulthood.

A feature common to a number of these factors is excessive oxidative stress. Spermatozoa are known to generate reactive oxygen species (ROS), which are believed to play an important role in normal sperm functions such as capacitation.[126,127] However, spermatozoa are particularly vulnerable to high levels of oxidative stress, which damage sperm structure (including damage to DNA) and impair function.[127-130] Excessive testicular levels of ROS are associated with varicocoele, infection, and inflammation[131] but also with lifestyle-linked influences such as tobacco and alcohol usage and obesity. Suffice it to say, testicular function seems to be sensitive to adverse events both pre- and post-natally. Some of the potentially more significant environmental stresses are briefly considered below.

3.3.1 | Environmental and lifestyle factors

Environmental influences and stresses

Air pollution is ubiquitous, and the WHO estimates that ~90% of people breathe air containing high levels of pollutants (https://www.who.int/airpollution/en/). Such pollution has been associated with a decrease in male fertility.[132] Possible mechanisms include hormonal changes due to endocrine disruption (see below), oxidative stress, cell DNA alteration, or epigenetic modifications, probably functioning in combination. Simultaneous exposure to several pollutants impedes identification of the impact of individual specific pollutants[133] and may result in synergistic interaction. Effects on regulation of scrotal temperature, for instance the consequences of sedentary occupations, might also be important. Application of scrotal heat stress in fertile men significantly decreased sperm concentration compared with pre-exposure values, as well as the percentages of motile and progressively motile spermatozoa and the proportion of morphologically normal spermatozoa. After removal of the heat stress, these parameters recovered to pre-exposure levels, the impact apparently being reversible.[134]

Smoking

Although the mechanisms underlying the effects of tobacco smoking remain uncertain, cigarette smoking is acknowledged to affect semen quality.[116,135,136] Li et al,[137] who undertook a large meta-analysis from 26 countries/regions (20 studies, 5865 participants), concluded that smoking causes a decline in semen quality in both fertile and infertile men. Subgroup analyses indicated that effect size was higher in infertile men than in the general population and that deterioration of semen quality is 'dose'-dependent, being more pronounced in moderate and heavy smokers (Sharma *et al*. 2016). A recent systematic review (16 studies; 10 823 infertile male participants [5257 smokers and 5566 non-smokers]) reached similar conclusions.[138] Oligozoospermia was significantly higher in smokers (RR: 1.29, 95% CI: 1.05-1.59), and normal sperm morphology was significantly lower in smokers (morphological defect RR: 2.44, 95% CI: 0.99-3.89). However, there was no apparent effect on motility.

Several cross-sectional studies show a significant effect of tobacco smoking on semen parameters and DNA fragmentation, as well as on gonadotrophin and testosterone levels.[139-143] Paternal smoking also significantly negatively influences ART outcomes including in IVF and ICSI.[144] Among former smokers, every additional year following smoking cessation by the male partner reduced the risk of ART failure by 4%, particularly miscarriage.[145] However, the data are not conclusive. For example, a prospective study on couples trying to conceive in the United States found little association between current male smoking or passive smoking in either partner, with reduced fecundability.[146] The Practice Committee of the ASRM, in their recent (2018) paper, concluded that 'smoking has not yet conclusively been shown to affect male fertility'.[147] Surprisingly, there are minimal data on the effect on semen parameters and fertility in men who have ceased smoking tobacco. Despite there being no absolute relationship between smoking tobacco and male infertility, available evidence supports a recommendation of smoking cessation and minimizing exposure to tobacco smoke among couples who are trying to conceive.

Electronic cigarettes (e-cigarettes) typically contain propylene glycol (a tasteless, odorless, colorless alcohol used in antifreeze), vegetable glycerin, a variable amount of nicotine, food grade flavoring, and water to generate an aerosol/vapor. Their use is commonly termed vaping and is generally viewed to be less harmful than conventional smoking. Nonetheless, studies in animal models show detrimental effects on spermatogenesis and increase in oxidative stress.[148,149] Although large-scale human studies are not yet available, recent reports of severe pulmonary disease associated with the use of electronic cigarettes (vaping) has raised significant concern about their short- and long-term safety.[150]

Caffeine

A recent systematic review of 28 papers (19 967 men in total) showed that semen parameters were not adversely affected by caffeine intake from coffee, tea, and cocoa drinks in most studies. However, a negative effect of cola-containing beverages and caffeine-containing soft drinks on semen volume, count, and concentration was observed. Caffeine intake appeared to be associated with aneuploidy and occurrence of DNA strand breaks. Coffee drinking in men was associated with prolonged time to pregnancy in some, but not all, studies.[151]

Alcohol

Given that a significant number of men regularly drink alcohol, it is perhaps surprising that the impact of alcohol consumption on male fertility is not well understood. Overall, alcohol consumption has been associated with lower semen volume but has a variable, and probably dose-dependent, impact on semen parameters.[137] Habitual alcohol consumption is associated with reduced semen quality and changes in reproductive hormones.[152] Similarly, semen volume, sperm count, motility, and number of morphologically normal spermatozoa were all significantly decreased in a study of those with heavy and chronic alcohol consumption.[153] In agreement with this, a recent meta-analysis indicates an effect of alcohol consumption on semen volume and sperm morphology. However, the review found no evidence for negative effects of occasional alcohol intake.[154]

Psychological stress

Psychological stress adversely affects testicular function such that stressful life events may be associated with decreased semen quality, an effect that might be primarily due to suppression of testosterone by raised corticosteroid levels.[155] Gollenberg et al[156] found that men reporting two or more recent stressful life events had an increased risk of having sperm concentration, motility, and morphology below 'normal' WHO thresholds. A meta-analysis of 57 cross-sectional studies (29 914 participants) indicated that psychological stress could lower sperm concentration and progressive motility, and increase the fraction of abnormal spermatozoa.[137] In a later cross-sectional study of 1215 Danish men, those with self-reported stress scores above an intermediate stress level had poorer semen quality and those with the highest stress levels had significantly lower sperm concentration, total sperm count, and seminal volume compared with those with intermediate stress levels.[157] An association has also been reported between stress/depression and semen quality for those experiencing fertility issues.[158] Antidepressant drugs used to treat depression, anxiety disorders, chronic pain, and a variety of other conditions have negative effects on sexual function and semen quality.[159] Nonetheless, non-pharmacological management of stress for infertile men, including cognitive behavior therapy, psychotherapy, and fertility counseling and support, may be beneficial.[160]

Sleep

Sleep disturbance is common, and its prevalence is increasing.[161] The influence of sleep (or sleep disturbance) on a range of physiological processes is well recognized and might contribute to male infertility.[162,163] Testosterone secretion follows a diurnal pattern, with rise in testosterone levels coinciding with rapid eye movement (REM) sleep rather than with changes in levels of melatonin.[164] Prolactin levels are also sleep-dependent, with an increase in prolactin secretion during sleep. Sleep duration is associated with testis size in healthy young men,[165] and obstructive sleep apnea, characterized by repetitive nocturnal hypoxia, is associated with increased levels of oxidative stress independent of obesity or reproductive hormone profile.[166] Combined or interacting effects of stress, depression, and poor sleep on semen parameters may be significant.[157,167-169]

Dietary intake and obesity

Diet, both in terms of nutritional balance and (in combination with inadequate levels of activity) excessive calorific intake leading to obesity, affects semen quality and male fertility. Diets high in processed meat, full-fat dairy products, alcohol, coffee, and sugar-sweetened beverages are associated with poor semen quality and lower fecundity rates.[170] Intake of full-fat dairy was inversely related to sperm motility and morphology among physically active men. Cheese, rather than overall dietary patterns, seemed to be primarily responsible for this effect, but the mechanism linking dairy food intake to lower sperm motility and morphology was not clear.[171] However,[162] reported lower sperm concentration and total sperm count were dose-dependently associated with high dietary intake of saturated fat among 701 young Danish men from the general population. The authors expressed concern that changes in diet over the past decades might in part be responsible for the high frequency of subnormal sperm counts reported.

The current increase in the incidence of obesity is also of concern with respect to male reproductive health. A meta-analysis of men from both the general population and from infertile couples showed the increased prevalence of azoospermia or oligozoospermia in men who were overweight or obese.[172] In a population-based prospective cohort of 501 couples, ejaculate volume showed an inverse linear relationship to body mass index and waist circumference. Similarly, total sperm count showed a significant negative linear association with waist circumference. The authors concluded that 'male infertility is a proxy of the overall male health status'.[173] Paternal obesity negatively affects both male fertility and assisted reproduction outcomes.[174] A recent, large-scale study (4440 men) showed statistically significant relationships between obesity and semen analysis parameters.[175] However, the effect of significant weight loss on semen analysis parameters is uncertain, and reports are conflicting, reporting either significant improvement,[176-180] no change,[181] or even deterioration.[182,183] It is certainly worth noting that weight loss intervention is complex and is likely to underlie the heterogeneity reported in these studies. It is also difficult to know whether observed improvements in semen quality are related to weight loss per se or to other, confounding factors such as change in diet, increase in exercise, and improved metabolic profiles.

Endocrine disruptors and male fertility

Endocrine-disrupting chemicals (EDCs) are 'exogenous substances, which have the ability to alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations'.[184-186] Known EDCs are a diverse range of small, often lipophilic, molecules that fall within many different and widely used categories of chemicals, for example, pesticides, pharmaceuticals, and plasticizers. Many of them are highly persistent and/or present at significant concentration in the environment such that humans are at high risk of exposure.[187] Even those that are rapidly metabolized, such as phthalates and bisphenol A, can be present in human tissues at significant levels due to continuous exposure. Many EDCs have been shown to interfere with

the activity of sex hormone steroids and thereby might affect functioning of the male reproductive system, for example, by acting as agonists or antagonists of the hormone receptors, by affecting the production or metabolism of endogenous sex steroids, or by modulating hormone signaling events in other ways.

Toxicological studies on animals indicate that some EDCs may indeed impair the development and functioning of the male reproductive system and thus might underlie, at least in part, the decline in human semen quality that has been observed in western populations ([70,189]; see Section 3.1). Sex differentiation of the male fetus seems to be particularly vulnerable to disruption of sex hormone function.[90,93,190] However, attempts to establish an association between levels of EDCs and semen quality in humans have proved difficult.[191] Wang et al[192] carried out a meta-analysis on association between EDC exposure and semen quality. Their analysis indicated that exposure to phthalates and organochlorine insecticides, for example, dichlorodiphenyl trichloroethane [DDT] might be associated with reduced semen quality. These studies focused on associations between adult exposure and semen quality, while toxicological data indicate that fetal EDC exposure, especially during sex differentiation, could have a lasting impact on adult reproductive function. Bonde et al[193] undertook a large meta-analysis (33 papers, 28 study populations) and investigated association between pre-natal and perinatal EDC levels and four aspects of male reproductive function: cryptorchidism, hypospadias, low sperm counts, and testicular cancer. A significant association of pre-natal and post-natal exposure to p,p′-DDE (p,p′-dichlorodiphenyldichloroethylene; a persistent metabolite of DDT) with the incidence of cryptorchidism, hypospadias, or testicular cancer was detected (13 studies). However, the overall odds ratio for the analysis (all exposures and outcomes) was 1.11 (95% CI 0.91-1.35). Di Nisio & Foresta[194] reviewed the data related to the effects of EDCs on male health and fertility, concentrating on phthalates, bisphenol A, organophosphate insecticides, perfluoroalkyls, and cadmium. They concluded that 'Despite promising discoveries, a causal relationship between reproductive disorders and exposure to specific toxicants has yet to be established.' Bliatka et al,[191] addressing the question of why clear evidence for the effects of EDCs on human male reproduction is still lacking, concluded that well-designed studies (case–control or cohort type) will be required. The timing of exposure in relation to relevant sensitive periods of development also needs to be considered. Furthermore, toxicological data have shown that exposures to combinations of EDCs, even if their initial site/mode of action varies, have effects that are additive or synergistic.[124,195] Simple dose–effect relationships of a single chemical may be masked by the concurrent exposure to other EDCs. Future studies should consider such combinations.

Anabolic-androgenic steroids

Anabolic-androgenic steroids (AAS) are non-prescription synthetic testosterone-like substances misused by athletes and bodybuilders.[196] At least 30 different AAS exist, including testosterone and its precursors (androstenedione, dehydroepiandrosterone, DHEA), 17α-alkyl-derivates (oral preparations, such as stanozolol, oxandrolone, and methyltestosterone), and 17β-ester-derivates (parenteral preparations, including nandrolone and testosterone esters).[197] They increase lean muscle mass in conjunction with weight training and are also used to improve strength and physical performance as well as exercise recovery. The vast majority of people who misuse steroids are male recreational power athletes in their 20s or 30s, with estimated 6.4% global lifetime prevalence rate.[198]

Simplistically, AAS use results in hypogonadotropic hypogonadism, resulting in decreased secretion of endogenous testosterone as well as oligozoospermia or azoospermia and testicular atrophy.[197] The effects are usually reversible.[199] However, it is notable that AAS users are more likely to take multiple supplements, including protein powders and creatine, other appearance- and performance-enhancing drugs (APED), and medication for erectile dysfunction [200] as well as recreational drugs.[201]

Lubricants

Although commonly available, studies have demonstrated most vaginal lubricants to be detrimental to spermatozoa in vitro.[202-204] Their impact on fertility and natural conception is unclear at best,[205] and however, the use of vaginal lubricants during intercourse should not be recommended for couples with infertility unless they are able to be deemed 'Sperm safe'.[206,207]

3.3.2 | Occupational risks to male fertility

Effects of occupational exposure

The effects on male fertility of occupational risks are poorly explored in clinical, for example, NICE or strategic guidelines,[1] yet there is a long history of research in this area. Most studies have addressed the question of occupational risk by examining semen quality as a surrogate marker of fertility of men in different occupations. Unfortunately, there is no consistency of assessment approach—for example, sperm concentration, motility, motile sperm concentration, and morphology have all been used as markers of fertility and data interpreted in different ways. There have also been studies examining the relationship between occupation and sperm DNA quality, but these are relatively rare and are similarly complicated by the array of different tests used (see section 2.3).

Occupational-related risks to fertility vary widely but can be simply classified into five types: physical agents (eg, noise, vibration, radiation, heat) and chemical agents (eg, fumes, gasses, aerosols, and mists); biological agents (eg, bacteria, fungi, insects, viruses); psychosocial (eg, stress, unsociable hours); Ergonomical (eg, manual handling, repetitive movement, working in confined spaces). While there are no a priori hypotheses to link many of these risks to poor male fertility, most studies have only concentrated on the effects of chemical exposures. The landmark study, linking chemical exposure with male infertility, published more than 40 years ago,

was the identification of 1,2-dibromo-3-chloropropane (DBCP) exposure as the cause of infertility in workers at a pesticide factory in California in 1977.[209,210] However, progress in identifying other chemical risks to male fertility was slow and by the early 1980s only five chemical risks had been identified: carbon disulfide, inorganic lead, 1,2-dibromo-3-chloropropane (as above), ethylene dibromide (with DBCP), and toluene diamine.[211] Subsequently, the risks of chemicals such as perchloroethylene, oxychlordane, and benzene were also identified.[212]

Perhaps the largest study to examine the relationship between occupation and semen quality was the Chemicals and Pregnancy Study (CHAPS-UK) which took place in the UK and examined the job history of nearly 2500 men attending 14 fertility clinics between 1999 and 2002. Analysis of the data from this study showed that: (a) moderate and high occupational exposure to glycol ether was related to low motile sperm count,[213] (b) poor sperm morphology was related to self-reported lifetime exposure to lead,[214] and (c) self-reported use of paint stripper and lead, but not glycol ether, were significantly related to both low motile sperm count and poor sperm morphology.[214] Interestingly, there was an increased risk of low motile sperm count[215] but not poor morphology[216] in men who were not working, suggesting a possible overall benefit to fertility of being in employment. While studies using measures of semen quality measures are relatively common, time to pregnancy (TTP) studies are rare. A meta-analysis of 23 studies suggested that TTP was increased following male partner exposure to lead and pesticides, with some evidence of harm following exposure to organic solvents or mixtures of oil products/chemicals.[217] The overall consensus from studies seems to be that from a public health perspective, there are relatively few occupational risks to the fertility of adult men in Western (post-industrial) societies,[218] though this conclusion remains tentative as large, comprehensive, robust studies using multivariate models remains to completed. It is also very important to point out that, in comparison, little is known about the risks to adult men in low and middle income countries.

Mitigation of occupational risk

A hierarchy of control measures to mitigate occupational risks has been developed. These measures include hazard limitation (remove the hazard from the workplace), substitution (replace the hazard with an alternative procedure/substance that is believed to mitigate the risk), engineering (isolate workers from the hazard), administrative (change working practices to minimize exposure), and Personal Protective Equipment (protect the worker by requiring that protective clothing or equipment is worn/used). There are relatively few documented examples of how these control measures have been used to mitigate occupational threats to male fertility. In the case of workers exposed to 1,2-dibromo-3-chloropropane (see above), while the first reports of effects on male fertility were published in 1977, it was not fully banned for agricultural use until 1985, and in 2009, it was still available in the supply chain. Advice to doctors about the clinical management of men working in high-risk occupations is generally poor. For example, National Institute for Health and Clinical Excellence advises that 'some occupations involve exposure to hazards that can reduce male or female fertility and therefore a specific enquiry about occupation should be made to people who are concerned about their fertility and appropriate advice should be offered.' However, there is no guidance about what the 'appropriate advice' should be.

4 | WORKSHOP SESSION 3—CLINICAL MANAGEMENT OF MALE INFERTILITY

4.1 | Genetic and epigenetic aspects of male infertility

Male infertility is a multifactorial condition in which the occurrence of known genetic factors is relatively frequent (eg, Klinefelter syndrome). The clinical significance of identifying genetic factors responsible for male infertility is important in the era of IVF and more particularly ICSI. Patients affected by a severe male factor condition who were previously infertile can now generate their own biological child. Counseling of the couple is an important requirement prior to IVF and in some instances includes testing of the female partner (for instance partner affected by congenital absence of vas deferens [CBAVD] due to cystic fibrosis transmembrane conductance regulator [CFTR] mutations).

4.1.1 | Genetic testing

In azoospermic men, the risk of being a carrier of known genetic anomalies is high (20%-25%), with the frequency progressively decreasing with increasing sperm output.[219-223] Genetic testing is therefore an essential tool for clinical decision-making. Identification of a causative genetic factor might allow prediction of outcome of testicular sperm retrieval, with the potential to avoid unnecessary surgical or medical treatments. It might also indicate the need for sperm cryopreservation in young adulthood if a progressive decrease in sperm production is predicted and/or highlight concerns regarding the patient's general health. Routine molecular genetic testing in men with quantitative impairment of spermatogenesis is currently limited to karyotype analysis and azoospermia factor (AZF) deletion screening. Chromosomal anomalies are the most frequent genetic factors in severe oligo/azoospermia. These tests are indicated in all patients affected by moderate or severe oligozoospermia and azoospermia.[220,224] Testing for AZF deletions has both diagnostic and prognostic value. Complete deletions of the AZFa and AZFb regions cause azoospermia with virtually no chance of sperm recovery through testis biopsy. It is important to note that the definition of the extension of AZFb deletion with a specific set of markers is necessary for prognostic purposes. Moreover, AZF deletions will be transmitted to the male offspring so clearly informed, and appropriate counseling of the couple is vital.[224]

4.1.2 | Mutation screening

Given the risk of transmitting genetic disorders, the diagnosis of known and the discovery of novel genetic factors in idiopathic infertility is of utmost clinical importance. Screening for mutations in candidate genes for specific male infertility phenotypes is not a routine practice but they are becoming increasingly available in a number of laboratories. Next-generation sequencing (NGS)-based panels are available for the following diseases: congenital hypogonadotropic hypogonadism (cHH), partial or mild androgen insensitivity syndrome, CBAVD and monomorphic forms of teratozoospermia, and asthenozoospermia.[219,220,222,223] Genome-wide association studies (GWAS) are based on high-throughput analyses (single nucleotide polymorphism [SNP] arrays and array comparative genomic hybridization [a-CGH]). Use of GWAS has not added clinically relevant data to our knowledge, strongly suggesting that common or low-frequency polymorphisms do not have a major role from a diagnostic perspective.[225] Only high-resolution X chromosome-specific a-CGH studies were able to identify clinically relevant deletions, the *TEX11* intragenic deletion, and *CNV67* (for reviews see[219,220,226]) Whole-exome sequencing has not proved to be a successful diagnostic tool in infertile patients with consanguineous parents or in familial cases of non-obstructive azoospermia (NOA), oligoasthenoteratozoospermia (OAT), monomorphic teratozoospermia, or asthenozoospermia.[219,220,227]

4.1.3 | Epigenetic effects

In addition to the effects of genetic mutations, the importance of epigenetic factors in the determination of semen quality is now recognized. Epigenetic alterations such as unequal protamine ratios, aberrant DNA methylation, histone modifications, and miRNA profile have been described in association with decreased semen quality, decreased fertilization ability, DNA damage, and reduced IVF success and embryo quality.[228] Aberrant methylation in imprinted genes could be responsible for imprinting diseases (Angelman and Beckwith–Wiedemann syndrome) in the offspring but this issue is still hotly debated. Diagnostic application of the acquired knowledge is still missing (methodological issues, lack of large studies,[229] and the clinical significance of these observations is yet to be established. Notwithstanding this, there is, at least in the animal literature, a rapidly growing awareness of the impact of paternal epigenetic transmission to the next generation.[230,231] The underlying mechanisms are still unclear, and the evidence in humans remains sparse, but this is an area of significant concern, and further research is clearly warranted.

4.1.4 | Future directions

Despite efforts and progress in understanding genetic defects underlying male factor infertility, large proportions of NOA and OAT cases are still defined as idiopathic. Exome/genome analysis is promising but the diagnostic role of these approaches in infertile patients with unrelated parents (the majority are idiopathic sporadic NOA or OAT) is still under evaluation. A comprehensive collection of functionally validated datasets and the development of new precise function predictor algorithms is needed to enable clinical interpretation of the observed variants (especially heterozygous autosomal variants) identified in sporadic idiopathic cases of NOA or OAT. This can be achieved through data sharing via large international consortia such as the GEMINI consortium (https://gemini.conradlab.org/) and the International Male Infertility Genomics Consortium (http://www.imigc.org/).

An emerging clinical issue is the higher rate of morbidity (including cancer) and lower life expectancy in infertile men (see section 3.2). The significantly higher deletion load in infertile men compared with normozoospermic men indicates a higher propensity to genomic instability in a subset of infertile men.[219,226] Furthermore, defects in mismatch repair proteins may lead to both spermatogenic failure and predisposition to cancer development.[232] This implies that infertile patients carrying specific genetic alterations need a lifelong follow-up to prevent or diagnose co-morbidities at an early stage (see above).

In addition to the effects of genetic mutations and epigenetic factors on male fertility, the role of gene–environmental interaction in male infertility is largely unexplored. Data on endocrine disrupters (see section 3.3.1 above) are still controversial, and the analysis of such an interaction has been performed mainly in animal models. The study of such effects in humans is highly challenging, but in the context of the apparent ongoing decline in semen quality (see section 3.1.1 above), this must be urgently explored.

4.2 | Hormonal treatment of male infertility

Despite progress in diagnosing the causes of infertility, in many cases, the etiology remains unknown, termed 'idiopathic infertility'.[233] In this category of patients, a number of empirical treatments have been proposed, including hormonal therapies, with controversial results.[222,234]

4.2.1 | Treatment of hypogonadotropic hypogonadism

Treatments for hypogonadotropic hypogonadism

Hypogonadotropic hypogonadism (HHG) is one of the few causes of male infertility where medical hormonal therapy is clearly effective. HHG is characterized by hypothalamic or pituitary dysfunction and low serum gonadotropin levels and reduced testicular function that presents clinically with testosterone deficiency, azoospermia, oligozoospermia, and/or small testicular volume. Clinical benefits have been reported for treatments with human chorionic gonadotropin (hCG) alone or in combination with urinary follicle-stimulating hormone (FSH[235,236]) Pulsatile gonadotropin-releasing hormone (GnRH) therapy seems to be the most physiological approach for

replacing hormones in HHG but, since it acts by inducing release of gonadotropic hormones from the anterior pituitary, it is a rational choice only for subjects with normal pituitary function.[237-239] Both pulsatile infusion of GnRH and combined gonadotropin and human menopausal gonadotropin (hCG/hMG) can effectively induce spermatogenesis in men with HHG, although it is not known which treatment regimen might be best.[240] Recombinant hCG[236] and LH[241] are also available, but their efficacy remains unknown. GnRH therapy is reported to induce a pregnancy rate of up to 80%, with a time to achieve pregnancy similar to combined gonadotropin therapy.[242] Worldwide, GnRH is less likely to be used than hCG alone or in combination with FSH due to cost, inconvenience and ineffectiveness in cases of panhypopituitarism or GnRH receptor mutation. A recent retrospective study[240] found that pulsatile GnRH therapy was associated with earlier spermatogenesis and larger testicular size compared with combined gonadotropin therapy. However, prospective randomized studies are required.

Use of FSH pre-treatment

The most important determinant of a successful response to gonadotropin therapy is baseline testis volume, higher volume being associated with a faster achievement of spermatogenesis.[242,243] The optimal regimen in patients with severe cases (testicular volume < 4 ml) is unknown. Fertility outcome is generally poorer in patients with signs of absent mini-puberty.[236,238] Pre-treatment with recombinant FSH prior to GnRH therapy (to mimic mini-puberty) may improve fertility outcomes and can be considered as an alternative to GnRH alone.[244] Data from animal studies indicate that a pre-pubertal 'window' occurs in the immature testis during which FSH can induce Sertoli cell proliferation.[245] Although use of FSH pre-treatment did increase sperm count and rate of conception compared with treatment with GnRH alone, the difference did not reach statistical significance and larger prospective studies are needed.

Recombinant human FSH (rhFSH) preparations seem to be as efficacious as the urinary derivatives,[233] with no difference in terms of stimulation of spermatogenesis or sperm concentration.[246] For better compliance, long-acting FSH formulations have been proposed (ie, corifollitropin alfa) in place of rhFSH,[247] which may improve patient satisfaction and compliance. Results have been encouraging, suggesting that, for men suffering from HHG who desire fertility, long-acting corifollitropin alfa can effectively and safely replace rhFSH in the treatment regimen. However, additional studies are needed. Comparative studies of different FSH preparations in males have not yet been thoroughly conducted. Moreover, there are no randomized controlled studies comparing gonadotropin treatment regimens.[246] The low prevalence of idiopathic HHG and other forms of HHG presents a significant obstacle to the organization of such trials and placebo-controlled studies, resulting in the absence of pubertal development in the control group, would be ethically unjustifiable.

After completion of therapy for infertility, most men with HHG will benefit from lifelong hormonal therapy.[248] This is usually achieved by switching to testosterone replacement. This will result in a return to clinical infertility, but it is more cost-effective than gonadotropin replacement therapy, and its use for long durations has been better characterized.[249] The best therapeutic choice needs to be elucidated for these vulnerable patients.

4.2.2 | Hormonal treatment of idiopathic infertility

The use of empirical medical therapies remains inconclusive and disappointing for men with idiopathic infertility. Estrogen receptor antagonists (eg, Tamoxifen, clomiphene) may be applied on the basis that a non-invasive treatment with limited side effects can be effective.[250] Aromatase inhibitors have also been recommended for men with impaired sperm parameters and a low testosterone/estrogen ratio but the data are limited.[251] RhFSH or purified human FSH application to idiopathic male infertility is more controversial.[250] Further evidence for the efficacy of and selection criteria for medical treatment of oligoasthenoteratozoospermia (OAT) is required.[250] The lack of clear consensus on the management of these conditions reflects our poor understanding of underlying etiology.

Finally, impaired semen quality may be associated with hypogonadism.[250] Treatment with exogenous testosterone is sometimes given but is not appropriate. Typical therapeutic doses of testosterone act as negative feedback on the hypothalamic–pituitary–gonadal axis, inhibiting secretion of LH and greatly reducing endogenous testosterone production.[207,250] If hypogonadism coincides with fertility issues, hCG treatment, which stimulates testosterone production of Leydig cells, could be considered, especially in men with low gonadotropins (secondary hypogonadism).

4.3 | Non-hormonal medical treatment of male infertility

In addition to the hormonal approaches outlined above, non-hormonal treatments have a role in clinical management of male infertility. Such treatments may effectively remove a reversible cause of infertility or can result in sufficient amelioration of the environment in which spermatozoa are produced and mature, that semen parameters are significantly improved. In cases such as idiopathic OAT or infertile patients without identifiable cause(s), non-hormonal treatments are frequently used, such as anti-inflammatory drugs and treatment with or enrichment of dietary antioxidant compounds and vitamins. Currently, the scientific evidence for the effectiveness of such empirical treatment is relatively poor and further studies are required to clarify the role of this approach.[252,253] However, three characterized, often co-existing conditions that interfere with reproductive function (oxidative stress, inflammation, and infection) may be responsive to focused (non-empirical) non-hormonal treatments.[254] These are the use of antioxidants (to ameliorate the damaging effects of excessive oxidative stress) and treatment with anti-inflammatory and antibiotic drugs.

4.3.1 | Antioxidants

There is a significant body of evidence to support the role of oxidative stress in sperm dysfunction,[126] and thus, antioxidant therapy appears a logical approach. A plethora of different products has been used clinically, and there are many, readily available, putative fertility-enhancing supplements on the market. However, critical evidence to support their use is absent (Martins da Silva 2019).[255]

A systematic review and meta-analysis of effect of nutrients and dietary supplements[256] found that sperm concentration was increased by selenium, zinc, omega-3 fatty acids, and coenzyme Q10 (CoQ10). Sperm counts were increased by omega-3 and CoQ10 supplementation. Selenium, zinc, omega-3, CoQ10, and carnitines increased sperm total motility, whereas sperm progressive motility was increased only after supplementation with carnitines. Finally, sperm morphology was enhanced by selenium, omega-3, CoQ10, and carnitine supplementation. Importantly, however male fertility was not assessed and the authors conclude that the results should be interpreted cautiously due to the limited sample size of the meta-analyzed studies and the considerable observed interstudy heterogeneity.[256]

A recent Cochrane review, which examined the role of supplementary oral antioxidants in subfertile men,[253] concluded that there was an increased live birth rate associated with antioxidant use for male subfertility (OR 1.79, 95% CI 1.20–2.67). However, the data were limited, being based on 7 randomized clinical trials, comprising 750 men and only 124 live births. A further 11 trials (786 men) indicate that antioxidants may increase clinical pregnancy rate (OR 2.97, 95% CI 1.91–4.63). However, when studies at significant risk of bias were removed from the analysis, there was no evidence of increased live birth rate. Overall, the evidence from these trials is low quality and the authors conclude that the data are inconclusive. While a healthy diet is certainly conducive to a healthy body and hence a potential association with gamete quality, we are a long way from concluding that supplementation of the diet is clearly beneficial for male fertility. Large randomized placebo-controlled trials are required to address these questions.[255]

4.3.2 | Anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAID) may have an impact on semen parameters. Some studies reported deleterious effect of salicylates and profens[257-259] on semen parameters. On the other hand, data on the effect of cox-2 inhibitors and sulfonanilides indicate positive effects.[260,261] Overall, it appears that, though NSAIDs might be useful for relief of symptoms in treating acute forms of male accessory gland inflammation, they should (if possible) be avoided for chronic usage. More studies are required to examine the positive effects of cox inhibitors on patients with 'idiopathic' leukocytospermia.[254]

Steroidal anti-inflammatory drugs

Steroidal anti-inflammatory drugs, such as glucocorticoids, have been used for the treatment of men with antisperm antibodies (ASA) but the results from RCTs have been mixed. Three showed no significant difference in pregnancy rates.[262-264] Two other RCTs showed a significant increase in pregnancy rate,[265,266] and a more recent RCT[267] on 241 men found that prednisolone treatment improved sperm motility/progressive motility and pregnancy rate in IVF (but not ICSI). However, before use, it is important to establish potential side effects of these treatments.

4.3.3 | Antibiotics

Where sub/infertility is associated with a clear bacterial infection (male accessory gland infection (MAGI), the use of antibiotics in treatment is well established. The choice of antibiotic prescribed is based up the nature of the microorganism identified (see review[254]).

Use of antibiotic treatment in men with leukocytospermia is much more contentious, particularly given global concerns of antimicrobial resistance (https://www.who.int/antimicrobial-resistance/global-action-plan/en/). A systematic review[268] identified only 11 RCTs concerning treatment of leukocytospermia performed over two decades (1984-2003).t The studies used variable methodology for leukocyte assessment and dose/duration of antibiotic intervention (quinolones, tetracyclines, macrolides, sulfonamides, and penicillins). Three of the trials showed statistically significant improvement of sperm concentration and/or motility between the treated and untreated groups,[269-271] but in three other studies no statistically significant differences were reported.[272-274] One trial reported significant improvement of sperm motility and morphology.[275] Antibiotics resulted in a statistically significant resolution of leukocytospermia in 5/8 trials.[270,271,276,277] The cure rate was reported in only 3/11 trials[270,271,273] but all studies showed a statistically significant decrease in the number of seminal bacteria. Four of the studies[270,271,276,277] reported the pregnancy rate after treatment but only one[271] showed a statistically significant improvement in the treated group compared with the untreated group. Overall, number of pregnancies was higher in the treatment groups. A complication for the use of antibiotics in treatment of male fertility is that there is evidence for adverse effects of antimicrobials on testis and sperm function. In summary, there are limited data available and there is a 'substantial need for well-designed clinical trials in this area'.[278-280]

5 | WORKSHOP SESSION 4—THE OVERUSE OF ICSI

There is absolutely no question that ICSI treatment for male factor infertility has transformed reproductive medicine and offers a clear option for treatment. However, there has been a significant

trend to use ICSI for non-male factor cases. For example, according to data from US National Assisted Technology Surveillance system (1996-2012), 65% of fresh cycles used ICSI yet only ~36% were reported male factor infertility.[281] Hans Evers highlighted the overuse of ICSI in a specific editorial entitled 'Santa Claus in the fertility clinic'.[282] Data from ICMART (International Committee for Monitoring Assisted Reproductive Technologies,[283] showed a substantial difference in the ICSI:IVF ratio from 1.4 in Asia to 60.3 in the Middle East. A recent ICMART report[5] using data from sixty-five countries and 2560 ART clinics showed that 'large disparities exist with ICSI used in nearly 97% of Middle East cycles compared with 55% in Asia and 69% in Europe'. The first report of ART in Africa[6] documented that 89% of aspirations were using ICSI (19,207/21,238 aspirations). According to the CDC, for women under 35 years of age, the majority of fresh, non-donor retrievals (68.9%) which used ICSI were 'without male factor'.[284] Clearly, ICSI use for non-male factor is widespread.

The justifications for the use of ICSI in non-male factor cases, for example, advanced maternal age, potential failed fertilization, unexplained infertility, cycles with few oocytes, and the counterarguments are well rehearsed.[282,285] However, it is important to recognize that the overwhelming majority of published data show no clinical benefit for ICSI use in non-male factor cases. For example, Li and colleagues examined a population-based cohort of 14 693 women in Victoria, Australia, between July 2009 and June 2014. They concluded that ICSI resulted in similar cumulative live birth rate compared with IVF for couples with non-male factor infertility.[286] More recently, Sustar et al[287] analyzed 3363 stimulated cycles (IVF = 1661, ICSI = 1702) and concluded that use of ICSI in normozoospermic men may result in lower clinical pregnancy and live birth rates.

Irrespective of this, there are key questions, as yet unanswered, that need to be addressed to minimize unnecessary treatment while maximizing success. Firstly, how do we appropriately select men with male factor infertility? Traditionally, male factor infertility has been diagnosed using basic semen parameters and the limitations of this approach are outlined above (section 2.1). To date, we have little data on more appropriate methods to diagnose the male (see section 2.2) and this makes the identification of those cases that are most suitable for ICSI very challenging and of course is a key argument used to justify ICSI for all. Nevertheless, it is critical we develop tools to diagnose the male in a more robust manner so that appropriate rational therapy can be applied. Furthermore, even when ICSI might be deemed appropriate, despite decades of research, we still have little idea how to select the best spermatozoon for injection.[52,288] Secondly, what are the long-term consequences of ICSI treatment? Even though initial data are relatively reassuring,[289,290] there is an urgent need, as expressed by the WHO Evidence Synthesis Group, to examine the long-term health outcomes of the children born from men with compromised fertility whatever the nature of the compromising event(s) (eg, genetics, environmental, iatrogenic, and/or occupational).[1] Moreover, when ICSI is used even though there is not a male factor issue, a fundamental question remains: Are there are any additional risks from the procedure itself[285,291]?

6 | DISCUSSION

The consensus group discussed the issue of semen analysis at length. It was agreed that basic semen analysis provides diagnostic but little functional information and that progress in assessment of sperm function over the last 25 years has been very limited and disappointing. Areas of major concern were the persistence of large variation between laboratories in the quality of semen analysis (including poor technical standards and failure to comply with WHO guidelines) and inconsistencies and confusion regarding interpretation of the data. The question of failure to follow WHO guidelines suggests that training and quality control measures are not effective. There is a real and immediate need to address this problem at national and international levels. Possibilities discussed included a regulatory requirement for trained andrologist in every clinic (which is already the case in some countries) and incorporating semen analysis standards into laboratory inspection and training. There was also an extended discussion about the use of WHO semen analysis reference ranges as cut-offs and the urgent need to accept and to explain to patients that semen analysis allows estimation of probability of successful conception/IVF fertilization but is very often not a yes/no diagnosis.

The discussion of functional tests mostly concerned the usefulness of those already available. Should tests of DNA fragmentation be used only in specific circumstances, for example patients who have had chemotherapy? Should such tests be used at all since there is no corrective treatment available? Some members of the group felt that patients wanted to know what was wrong—whereas others reported that patients attending their clinic just want a healthy child. As with semen analysis, there is interlaboratory variation in testing and there might be benefit in having regional, specialized testing centers. There was universal agreement that development of simple, cost-effective functional tests should continue to be an objective for researchers and industry, particularly aiming for accuracy and ease of use through automation. Furthermore, since most tests are destructive (providing information about the sample population but not about the actual cell(s) tested), development of non-destructive tests that provide information on the status of an individual cell would be extremely valuable.

There was acceptance that the evidence for a progressive fall in human sperm counts was becoming much stronger and that there was a need for further research, both to widen the range of populations investigated (nearly all data currently concern western men) and to resolve doubts regarding the nature of the observed trend. In particular, prospective studies should be carried out. Another significant point raised in discussion was that the data suggest that birth year is more important than year of testing, suggesting that the factors responsible for the observed decline might exert major effects on development of testicular capacity in utero. A number of issues discussed related to both environmental/lifestyle influences

and occupational exposure, including the importance of realizing that exposures are often to mixtures of chemicals/toxicants and that significant interactions and synergistic effects might occur. Also, the effects of exposures to these compounds must be considered within their genetic background, both when developing hypotheses and designing studies, which will require global collaboration. Another area of significant discussion was the need for balanced communication of concerns to governments and to the public—which requires use of the 'collaborative engagement' model. Surprisingly, there are very few examples of a coordinated approach to male reproductive health. The best example is that of, 'Healthy Male' (formerly Andrology Australia https://www.healthymale.org.au/) coordinated by the Australian Government. Unfortunately, other governments/national societies have not followed this comprehensive approach.

A key factor identified during the discussion was the lack of a quantitative, detailed assessment of the economic, and societal burden of male reproductive health. For almost all other diseases, compelling evidence of economic effect has been presented, but no such analysis has been undertaken for male reproductive health.[2] An accurate economic impact assessment is fundamental to underpin scientific arguments and modify policy. A good example of such analysis is that of weather forecasting. Alley and colleagues argued that investment in accurate weather prediction models paid economic dividends ranging from 3 to 10 times the amount invested.[292] The World Bank report on funding of weather forecasting suggested that meeting a worldwide investment need of 1.5-2 US$ billion and ongoing annual costs of ~500 million could save 23 000 lives per year and would achieve up to US$ 30 billion per year in economic benefits.[292] These are powerful statements that help highlight the impact of a discipline to policymakers.

The consensus group agreed that advice about the effects of life exposures on fertility had value but also felt that, although there was clear evidence for effects of lifestyle/behavior prior to attempting to conceive, the evidence for improvement after intervention, for example giving up smoking was poor. More detailed and powerful studies on such interventions are needed. Similarly, it was felt that more evidence is needed to guide the use of therapies such as antioxidants for the reduction of oxidative stress. Could there be a subpopulation that would benefit from such therapy (potentially related to genetic background) and might there even be a subpopulation for whom free radical levels were already unusually low such that they should not be further lowered? Development of robust routine testing of semen for free radicals/oxidative stress is needed.

A fundamental point that was raised in many of the presentations and throughout the discussion was the need, in almost all areas, for large, high-quality robust studies, including well-controlled RCTs.

Following presentation of evidence, debate and discussion the group identified a series of 36 consensus points. One important objective of providing these is for them to provide clear statements of evidence gaps and/or potential future research areas/topics. Appropriate funding streams addressing these can then be prioritized, and consequently, in the short and medium term, answers can be provided. By using this strategic approach, our discipline can make the rapid progress necessary to address key scientific, clinical, and societal questions that do and will face us.

7 | CONSENSUS POINTS

1. Male subfertility is a complex and heterogeneous condition. The development of robust diagnostics and treatment interventions should ultimately allow development of individualized approaches to patient care.
2. A detailed economic impact assessment of the costs of male subfertility and infertility is urgently needed.
3. There is a need for prospective collection of quality semen analysis data from global sources to improve clinical and health policy decision-making.
4. Threshold values for semen analysis have an acceptable specificity but low sensitivity for identifying male (sub)fertility. Therefore, caution should be used in the interpretation of results in relation to the WHO manual reference values.
5. Semen quality is not just a matter of reproductive potential. There is an emerging clinical issue related to the higher morbidity (including cancer) and lower life expectancy in subfertile men relative to the general population. Recent studies have revealed genetic links in some cases. The identification of co-morbidities needs to be the subject of prospective studies.
6. Interpretation of semen analysis results will be guided by the endpoint of interest.
7. Interpretation of semen analysis for diagnosis of male subfertility is more predictive when several semen characteristics are considered together.
8. There is a clear need for training and education, at the national and international level, of laboratory personnel performing semen analysis, leading to individual certification. This requires effective maintenance of competence, internal quality control, and external quality assurance programs.
9. There is a need to develop accurate, automated methods for semen analysis and testing sperm functional potential. This could include development of point-of-care devices that have been fully validated and include accompanying QC/QA tools.
10. Definitions of the specific quality expectations and performance of QC/QA programs in laboratory andrology are needed.
11. At present, most sperm function tests are too technically complex and/or expensive for routine use. Simpler, robust, cost-effective tests are needed, with validated, prospective clinical endpoint data to support the interpretation of results.
12. Sperm DNA fragmentation is higher in some men with subfertility. However, the range of tests and variations in protocols, as well as a general lack of test-specific validated threshold values, continues to limit its potential clinical utility. Large-scale, prospective clinical studies are needed.
13. Research efforts should be focused on the development of non-destructive tests of sperm function that allow the tested sperm to be selected and used in the attempt to establish a pregnancy.

14. The studies that show a decline in sperm count raise concerns with regard to future male fertility and health. However, they are potentially influenced by the assessment methods that were used. Therefore, prospective studies using rigorously standardized methodology are warranted.
15. These prospective studies should take into account that the birth year is likely to have more influence on the result than the year in which the test was performed. This is because it is likely that in utero exposures influence testicular functional capacity that can then be further affected by personal and environmental exposures.
16. Male reproductive health studies that take into account the interaction between environmental exposures and genetic background should be conducted.
17. High-quality epidemiological studies in diverse populations are needed to better identify environmental and occupational risks to male reproductive health.
18. Mixtures of chemicals and additional exposures should be taken into consideration when studying their effect(s) on male reproductive health. Caution is urged in relation to studies of individual chemicals or single exposures.
19. Pharmaceutical reference sources need to include more information on potential adverse effects on male reproductive health.
20. There is a need to develop effective methods and engagement strategies to provide information about male reproductive health to the public, regulators, and policymakers.
21. There are few effective medical interventions for male infertility. Hypogonadotropic hypogonadism is a well-defined condition that responds to hormonal treatment.
22. In general, there is no good quality evidence to support empirical hormonal treatment for idiopathic oligozoospermia or unexplained male subfertility. Large-scale studies are needed to identify who could benefit from this type of treatment; a clinical endpoint of such studies should be live birth rate.
23. Exogenous testosterone treatment should not be used in an attempt to improve sperm production as it has a negative effect on spermatogenesis.
24. Since medical professional societies have already addressed the controversial topic of varicocoele treatment, the group elected not to consider it further.
25. TESE and micro-TESE are ways of obtaining spermatozoa in some, but not all men with non-obstructive azoospermia. Only a few markers are known to predict poor TESE outcome (eg, Y-chromosome microdeletions), and therefore, additional markers are needed to identify those who would benefit from this procedure.
26. A better understanding of the etiology of spermatogenic arrest is needed to identify and develop treatments for affected men.
27. Future research should focus on finding simple, cost-effective screening technologies for male genital tract infection.
28. Smoking negatively affects personal and reproductive health. Patients should be offered smoking cessation strategies to minimize exposure to tobacco smoke and vaping.
29. Oxidative stress is a recognized pathology in male subfertility. Dietary and vitamin supplements appear to improve semen quality, but the data are unclear as to whether antioxidant supplementation is an effective intervention. Further, well-designed, large-scale randomized controlled trials are needed.
30. The use of intimate lubricants should be recommended with caution since many are toxic to sperm. If a coital lubricant is needed, products established as being 'sperm friendly' should be used.
31. Genetic testing in azoospermia, severe oligozoospermia, total lack of sperm motility, and monomorphic teratozoospermia has diagnostic and prognostic value and is important for genetic counseling.
32. With the current diagnostic tests, there is still a large proportion of idiopathic NOA/OAT cases who are likely to be affected by undefined genetic factors. Exome/genome analysis studies have the potential to develop a gene panel to enhance diagnosis and pre-TESE prognosis in such men.
33. The current data do not support the use of ICSI for non-male factor patients.
34. Not all cases of male factor infertility justify treatment by ICSI; some could be treated using IUI or IVF.
35. The potential risks of the indiscriminate use of ICSI to the health of children have yet to be firmly established. High-quality longitudinal studies are therefore required.
36. Given concerns related to the indiscriminate use of ICSI, more research is required to better understand the sperm's contribution to fertilization and early embryonic development.

ACKNOWLEDGEMENTS

The authors wish to thank Anna-Maria Andersson from NJ laboratory who assisted on the endocrine disrupting chemicals section.

CONFLICT OF INTEREST

AP is the Editor in Chief of Human Fertility, a Trustee of the Progress Educational Trust and Chairman of the advisory committee of the UK National External Quality Assurance Schemes in Andrology (all unpaid). In the last 24 months, he has undertaken paid consultancy, Speaker fees or Contributor fees for the British Broadcasting Corporation, CamNutra Ltd, Cryos, Ferring Pharmaceuticals A/S, Pharmasure Ltd but all monies associated with this are paid to The University of Sheffield. His research laboratory has received grant funding from Medical Research Council, National Institute for Health Research and Weston Park Cancer Charity. CB is the editor of RBMO, has received lecturing fees from Merck, Pharmasure, and Ferring, and was on the Scientific Advisory Panel for Ohana BioSciences (2018). CLRB was chair of the World Health Organization Expert Synthesis Group on Diagnosis of Male infertility (2012-2016). His laboratory receives funding from University of Dundee, Astra Zeneca, Genus, MRC, NHS Tayside, Bill, and Melinda Gates Foundation and Chief Scientist Office/NHS research Scotland. DM has undertaken consulting work since 1986 and has been a full-time freelance consultant since October 1999. He is currently President and co-owner of

Oozoa Biomedical Inc, a Vancouver-based international consulting company providing services in the reproductive biomedicine field since March 2000. He has performed work, on either commercial or a pro bono basis, for many clients and groups including the following: assisted conception clinics and sperm banks; biotechnology, pharmaceutical and ART products companies; academic institutions; researchers; government agencies; non-government organizations; professional associations and other bodies. No commercial or financial interest influenced any contribution made during the workshop or in preparing the Report. SM has undertaken consulting work since 1988 and has been a full-time freelance consultant since April 2010. She is currently a Director and co-owner of Oozoa Biomedical Inc, a Vancouver-based international consulting company providing services in the reproductive biomedicine field since March 2000. She has performed work, on either commercial or a pro bono basis, for many clients and groups including: assisted conception clinics and sperm banks; biotechnology, pharmaceutical and ART products companies; academic institutions; researchers; government agencies; non-government organizations; professional associations and other bodies. No commercial or financial interest influenced any contribution made during the workshop or in preparing the Report. MA, EB, MF, CDJ, NJ, CK, AM, SMDS, and SP report no conflict of interest.

AUTHORS' CONTRIBUTIONS

Christopher LR Barratt and David Mortimer where Co-conveners of the workshop. Mohamed Fawzy and Ali Mahran are UEARS co-founders and representatives in addition to their scientific contributions throughout the workshop. Each author prepared a precis with references for their individual presentation including potential consensus points. All contributors actively took part in discussions, and the consensus points were arrived at after full and open discussion. SJP and CLRB drafted the manuscript based on the materials provided by contributors. All authors contributed to the writing and approval of the final article.

REFERENCES

1. Barratt CLR, Björndahl L, De Jonge CJ, et al. The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. *Hum Reprod Update*. 2017;23:660-680.
2. Barratt CLR, De Jonge CJ, Sharpe RM. 'Man Up': the importance and strategy for placing male reproductive health centre stage in the political and research agenda. *Hum Reprod* 2018;33:541-545.
3. Baker K, Li J, Sabanegh E Jr. Analysis of semen parameters in male referrals: impact of reference limits, stratification by fertility categories, predictors of change, and comparison of normal semen parameters in subfertile couples. *Fertil Steril*. 2015;103:59-65.
4. De Jonge C, Barratt CLR. The present crisis in male reproductive health: an urgent need for a political, social, and research roadmap. *Andrology*. 2019;7:762-768. https://doi.org/10.1111/andr.12673.
5. Adamson GD, de Mouzon J, Chambers GM, et al. International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology. *Fertil Steril*. 2011;110:1067-1080.
6. Dyer S, Archary P, de Mouzon J, Fiadjoe M, Ashiru O, African N. Assisted reproductive technologies in Africa: first results from the African Network and Registry for Assisted Reproductive Technology, 2013. *Reprod Biomed Online*. 2019;38:216-224.
7. Aitken RJ. Not every sperm is sacred; a perspective on male infertility. *Mol Hum Reprod*. 2018;24:287-298.
8. Macomber D, Sanders MB. The spermatozoa count Its value in the diagnosis, prognosis and treatment of sterility. *New Engl J Med*. 1929;200:981-984.
9. Macleod J, Gold RZ. The male factor in fertility and infertility II Spermatozoon counts in 1000 men of known fertility and in 1000 cases of infertile marriage. *J Urol*. 1951;66:436-449.
10. Belsey MA, Moghissi KS, Eliasson R, Paulsen CA, Gallegos AJ, Prasad MR. *Laboratory manual for the examination of human semen and semen-cervical mucus interaction*. Singapore: Press Concern; 1980.
11. Merino G, Carranza-Lira S. Infection and male infertility: effect of different antibiotic regimens on semen quality. *Arch Androl*. 1995;35:209-212.
12. Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*. 2010;16:231-245.
13. World Health Organization. *WHO laboratory manual for the examination and processing of human semen*, 5th edn. World Health Organization; 2010. ISBN 978 92 4 154778 9.
14. Bonde JP, Ernst E, Jensen TK, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet*. 1998;352:1172-1177.
15. Guzick DS, Overstreet JW, Factor-Litvak P, et al. Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 2001; 345: 1388-1393.
16. Slama R, Eustache F, Ducot B, et al. Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Hum Reprod*. 2002;17:503-515.
17. Patel AS, Leong JY, Ramasamy R. Prediction of male infertility by the World Health Organization laboratory manual for assessment of semen analysis: A systematic review *Arab. J Urol*. 2018;16:96-102.
18. Björndahl L. What is normal semen quality? On the use and abuse of reference limits for the interpretation of semen analysis results. *Hum Fertil (Camb)*. 2011;14:179-186.
19. Murray KS, James A, McGeady JB, Reed ML, Kuang WW. Nangia AK The effect of the new 2010 World Health Organization criteria for semen analyses on male infertility. *Fertil Steril*. 2012;98:1428-1431.
20. Björndahl L, Barratt CL, Mortimer D, Jouannet P. 'How to count sperm properly': checklist for acceptability of studies based on human semen analysis. *Hum Reprod*. 2016;31:227-232.
21. Carrell DT, De Jonge CJ. The troubling state of the semen analysis. *Andrology*. 2016;4:761-762.
22. Nieschlag E, Pock T, Hellenkemper B. External quality control of semen analysis reveals low compliance with WHO guidelines. *J Reproduktionsmed Endokrinol Online*. 2017;14:306-310.
23. Rohayem J, Hauffa BP, Zacharin M, Kliesch S, Zitzmann M. Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? -a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. *Clin Endocrinol (Oxf)* 2017; 86: 75-87.
24. Björndahl L, Barratt CL, Fraser LR, Kvist U, Mortimer D. ESHRE basic semen analysis courses 1995–1999: immediate beneficial effects of standardized training. *Hum Reprod*. 2002;17:1299-1305.
25. Franken DR. Semen analysis workshops in India and Africa: the vital role of training and external quality control programmes. *Facts Views Vis Obgyn*. 2013;5:100-105.

26. Walczak-Jedrzejowska R, Marchlewska K, Oszukowska E, Filipiak E, Bergier L, Slowikowska-Hilczer J. Semen analysis standardization: is there any problem in Polish laboratories? *Asian J Androl*. 2013;15:616-621.
27. Filimberti E, Degl'Innocenti S, Borsotti M, et al. High variability in results of semen analysis in andrology laboratories in Tuscany (Italy): the experience of an external quality control (EQC) programme. *Andrology*. 2013;1:401-407.
28. Punjabi U, Wyns C, Mahmoud A, Vernelen K, China B, Verheyen G. Fifteen years of Belgian experience with external quality assessment of semen analysis. *Andrology*. 2016;4:1084-1093.
29. Whitten SJ, Nangia AK, Kolettis PN. Select patients with hypogonadotropic hypogonadism may respond to treatment with clomiphene citrate. *Fertil Steril*. 2006;86:1664-1668.
30. Publicover S, Harper CV, Barratt C. [Ca2+]i signalling in sperm–making the most of what you've got. *Nat Cell Biol*. 2007;9:235-242.
31. Oehninger S, Franken DR, Ombelet W. Sperm functional tests. *Fertil Steril*. 2014;102:1528-1533.
32. Oehninger S, Ombelet W. Limits of current male fertility testing. *Fertil Steril*. 2019;111:835-841.
33. Xu F, Guo G, Zhu W, Fan L. Human sperm acrosome function assays are predictive of fertilization rate in vitro: a retrospective cohort study and meta-analysis. *Reprod Biol Endocrinol*. 2018;16:81.
34. Mortimer ST, van der Horst G, Mortimer D. The future of computer-aided sperm analysis. *Asian J Androl*. 2015;17:545-553.
35. Mortimer D, Mortimer ST. Computer-Aided Sperm Analysis (CASA) of sperm motility and hyperactivation. *Methods Mol Biol*. 2013;927:77-87.
36. Camargo M, Intasqui P, Bertolla RP. Understanding the seminal plasma proteome and its role in male fertility. *Basic Clin Androl*. 2018;28:6.
37. Amaral A, Lourenco B, Marques M, Ramalho-Santos J. Mitochondria functionality and sperm quality. *Reproduction*. 2013;146:R163-R174.
38. Calvert SJ, Reynolds S, Paley MN, Walters SJ, Pacey A. Probing human sperm metabolism using 13C-magnetic resonance spectroscopy. *Mol Hum Reprod* 2019;25:30-41.
39. Reynolds S, Calvert SJ, Paley MN, Pacey AA. 1H Magnetic Resonance Spectroscopy of live human sperm. *Mol Hum Reprod*. 2017;23:441-451.
40. Schinfeld J, Sharara F, Morris R, et al. Cap-Score prospectively predicts probability of pregnancy. *Mol Reprod Dev*. 2018;85:654-664.
41. Pena FJ, Ortiz Rodriguez JM, Gil MC, Ortega Ferrusola C. Flow cytometry analysis of spermatozoa: Is it time for flow spermetry? *Reprod Domest Anim*. 2018;53(Suppl 2):37-45.
42. Espinoza JA, Schulz MA, Sanchez R, Villegas JV. Integrity of mitochondrial membrane potential reflects human sperm quality. *Andrologia*. 2009;41:51-54.
43. Zhang G, Wang Z, Ling X, et al. Mitochondrial biomarkers reflect semen quality: results from the MARCHS study in Chongqing, China. *PLoS ONE*. 2016;11:e0168823.
44. Cardona C, Neri QV, Simpson AJ, et al. Localization patterns of the ganglioside GM1 in human sperm are indicative of male fertility and independent of traditional semen measures. *Mol Reprod Dev*. 2017;84:423-435.
45. Krausz C, Bonaccorsi L, Luconi M, et al. Intracellular calcium increase and acrosome reaction in response to progesterone in human spermatozoa are correlated with in-vitro fertilization. *Hum Reprod*. 1995;10:120-124.
46. Krausz C, Bonaccorsi L, Maggio P, et al. Two functional assays of sperm responsiveness to progesterone and their values in in-vitro fertilization. *Hum Reprod*. 1996;11:1661-1667.
47. Alasmari W, Barratt CLR, Publicover S, et al. The incidence and clinical significance of defects in calcium signalling pathways mediating human sperm hyperactivation in donors and sub fertile patients. *Hum Reprod*. 2013;28:866-876.
48. Kelly MC, Brown SG, Costello SM, et al. Single-cell analysis of [Ca2+]i signalling in sub-fertile men: characteristics and relation to fertilization outcome. *Hum Reprod*. 2018;33:1023-1033.
49. Williams HL, Mansell S, Alasmari W, et al. Specific loss of CatSper function is sufficient to compromise fertilizing capacity of human spermatozoa. *Hum Reprod*. 2015;30:2737-2746.
50. Brown SG, Publicover SJ, Mansell SA, et al. Depolarization of sperm membrane potential is a common feature of men with subfertility and is associated with low fertilization rate at IVF. *Hum Reprod*. 2016;31:1147-1157.
51. Mortimer D. Structured management as a basis for cost-effective infertility care. In: Gagnon C, ed. *The Male Gamete: From Basic Knowledge to Clinical Applications*. Vienna, IL: Cache River Press; 1999: 363-370.
52. Sakkas D, Ramalingam M, Garrido N, Barratt CL. Sperm selection in natural conception: what can we learn from Mother Nature to improve assisted reproduction outcomes? *Hum Reprod Update*. 2015;21:711-726.
53. Evgeni E, Charalabopoulos K, Asimakopoulos B. Human sperm DNA fragmentation and its correlation with conventional semen parameters. *J Reprod Infertil*. 2014;15:2-14.
54. Muratori M, Marchiani S, Tamburrino L, et al. DNA fragmentation in brighter sperm predicts male fertility independently from age and semen parameters. *Fertil Steril*. 2015;104:582-590.
55. Santi D, Spaggiari G, Simoni M. Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management – meta-analyses. *Reprod Biomed Online*. 2018;37:315-326.
56. Cissen M, Wely MV, Scholten I, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. *PLoS ONE*. 2016;11:e0165125.
57. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. *Fertil Steril*. 2019;112:54-60.
58. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. *Reprod Biomed Online*. 2015;30:120-127.
59. Robinson L, Gallos ID, Conner SJ, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Hum Reprod*. 2012;27:2908-2917.
60. Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian J Androl*. 2017;19:80-90.
61. Tan J, Taskin O, Albert A, Bedaiwy MA. Association between sperm DNA fragmentation and idiopathic recurrent pregnancy loss: a systematic review and meta-analysis. *Reprod Biomed Online*. 2019;38:951-960.
62. Aitken RJ, Finnie JM, Muscio L, et al. Potential importance of transition metals in the induction of DNA damage by sperm preparation media. *Hum Reprod*. 2014;29:2136-2147.
63. Muratori M, Tarozzi N, Cambi M, et al. Variation of DNA fragmentation levels during density gradient sperm selection for assisted reproduction techniques: a possible new male predictive parameter of pregnancy? *Medicine (Baltimore)*. 2016;95:e3624.
64. Muratori M, Tarozzi N, Carpentiero F, et al. Sperm selection with density gradient centrifugation and swim up: effect on DNA fragmentation in viable spermatozoa. *Sci Rep*. 2019;9:7492.
65. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? *Syst Biol Reprod Med*. 2011;57:78-85.
66. Chen Q, Zhao JY, Xue X, Zhu GX. The association between sperm DNA fragmentation and reproductive outcomes

following intrauterine insemination, a meta analysis. *Reprod Toxicol*. 2019;86:50-55.
67. Capogrosso P, Ventimiglia E, Boeri L, et al. Male infertility as a proxy of the overall male health status. *Minerva Urol Nefrol*. 2018;70:286-299.
68. Hanson BM, Eisenberg ML, Hotaling JM. Male infertility: a biomarker of individual and familial cancer risk. *Fertil Steril*. 2018;109:6-19.
69. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ*. 1992;305:609-613.
70. Levine H, Jorgensen N, Martino-Andrade A, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update*. 2017;23:646-659.
71. MacLeod J, Wang Y. Male fertility potential in terms of semen quality: a review of the past, a study of the present. *Fertil Steril*. 1979;31:103-116.
72. Nelson CM, Bunge RG. Semen analysis: evidence for changing parameters of male fertility potential. *Fertil Steril*. 1974;25:503-507.
73. Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. *Environ Health Perspect*. 1997;105:1228-1232.
74. Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med*. 1995;332:281-285.
75. Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ*. 1996;312:467-471.
76. Splingart C, Frapsauce C, Veau S, Barthelemy C, Royere D, Guerif F. Semen variation in a population of fertile donors: evaluation in a French centre over a 34-year period. *Int J Androl*. 2012;35:467-474.
77. Mendiola J, Jorgensen N, Minguez-Alarcon L, et al. Sperm counts may have declined in young university students in Southern Spain. *Andrology*. 2013;1:408-413.
78. Axelsson J, Rylander L, Rignell-Hydbom A, Giwercman A. No secular trend over the last decade in sperm counts among Swedish men from the general population. *Hum Reprod*. 2011;26:1012-1016.
79. Priskorn L, Nordkap L, Bang AK, et al. Average sperm count remains unchanged despite reduction in maternal smoking: results from a large cross-sectional study with annual investigations over 21 years. *Hum Reprod*. 2018;33:998-1008.
80. Jorgensen N, Joensen UN, Jensen TK, et al. Human semen quality in the new millennium: a prospective cross-sectional population-based study of 4867 men. *BMJ Open*. 2012;2:e000990.
81. Vierula M, Niemi M, Keiski A, Saaranen M, Saarikoski S, Suominen J. High and unchanged sperm counts of Finnish men. *Int J Androl*. 1996;19:11-17.
82. Jorgensen N, Vierula M, Jacobsen R, et al. Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl*. 2011;34:e37-48.
83. Rodprasert W, Virtanen HE, Sadov S, et al. An update on semen quality among young Finnish men and comparison with Danish data. *Andrology*. 2019;7:15-23.
84. Sengupta P, Nwagha DS, et al. Evidence for decreasing sperm count in African population from 1965 to 2015. *African Health Sciences*. 2017;17:418-427.
85. Mishra P, Negi MPS, Srivastava M, Singh K, Rajender S. Decline in semen quality in Indian men over the last 37 years. *Reprod Biol Endocrinol*. 2018;16:103.
86. Huang C, Li B, Zu K, et al. Decline in semen quality among 30,636 young Chinese men from 2001–2015. *Fertil Steril*. 2017;104:83-88.
87. Wang L, Zhang L, Song XH, Zhang HB, Xu CY, Chen ZJ. Decline of semen quality among Chinese sperm bank donors within 7 years (2008–2014). *Asian J Androl*. 2017;19:521-525.
88. Yuan H-F, Shangguan H-F, Zheng Y, et al. Decline in semen concentration of healthy Chinese adults: evidence from 9357 participants from 2010 to 2015. *Asian J Androl*. 2018;20:379-384.
89. Christensen P, Stryhn H, Hansen C. Discrepencies in the determination of sperm concentration using Bürker-Türk, Thoma and Makler counting chambers. *Theriogenology*. 2005;63:992-1003.
90. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod*. 2001;16:972-978.
91. Jorgensen N, Rajpert-De Meyts E, Main KM, Skakkebaek NE. Testicular dysgenesis syndrome comprises some but not all cases of hypospadias and impaired spermatogenesis. *Int J Androl*. 2010;33:298-303.
92. Nordkap L, Joensen UN, Blomberg Jensen M, Jorgensen N. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. *Mol Cell Endocrinol*. 2012;355:221-230.
93. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, et al. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol Rev*. 2016;96:55-97.
94. Jorgensen N, Carlsen E, Nermoen I, et al. East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Hum Reprod*. 2002;17:2199-2208.
95. Fernandez MF, Duran I, Olea N, et al. Semen quality and reproductive hormone levels in men from Southern Spain. *Int J Androl*. 2012;35:1-10.
96. Iwamoto T, Nozawa S, Mieno MN, et al. Semen quality of 1559 young men from four cities in Japan: a cross-sectional population-based study. *BMJ Open*. 2013;3:e002222.
97. Mendiola J, Stahlhut RW, Jorgensen N, Liu F, Swan SH. Shorter anogenital distance predicts poorer semen quality in young men in Rochester. *New York Environ Health Perspect*. 2011;119:958-963.
98. Paasch U, Salzbrunn A, Glander HJ, et al. Semen quality in sub-fertile range for a significant proportion of young men from the general German population: a co-ordinated, controlled study of 791 men from Hamburg and Leipzig. *Int J Androl*. 2008;31:93-102.
99. Punab M, Zilaitiene B, Jorgensen N, et al. Regional differences in semen qualities in the Baltic region. *Int J Androl*. 2002;25:243-252.
100. Iwamoto T, Nozawa S, Yoshiike M, et al. Semen quality of fertile Japanese men: a cross-sectional population-based study of 792 men. *BMJ Open*. 2013;3:e002223.
101. Jorgensen N, Andersen AG, Eustache F, et al. Regional differences in semen quality in Europe. *Hum Reprod*. 2001;16:1012-1019.
102. Swan SH, Brazil C, Drobnis EZ, et al. Geographic differences in semen quality of fertile US males. *Environ Health Perspect*. 2003;111:414-420.
103. Eustache F, Auger J, Cabrol D, Jouannet P. Are volunteers delivering semen samples in fertility studies a biased population? *Hum Reprod*. 2004;19:2831-2837.
104. Sanchez-Pozo MC, Mendiola J, Serrano M, et al. Proposal of guidelines for the appraisal of SEMen QUAlity studies (SEMQUA). *Hum Reprod* 2013; 28: 10-21.
105. Jorgensen N, Asklund C, Carlsen E, Skakkebaek NE. Coordinated European investigations of semen quality: results from studies of Scandinavian young men is a matter of concern. *Int J Androl*. 2006;29:54-61; discussion 105–108.
106. Smarr MM, Sapra KJ, Gemmill A, et al. Is human fecundity changing? A discussion of research and data gaps precluding us from having an answer. *Hum Reprod*. 2017;32:499-504.
107. Eisenberg ML, Li S, Behr B, et al. Semen quality, infertility and mortality in the USA. *Hum Reprod*. 2014;29:1567-1574.

108. Eisenberg ML, Li S, Behr B, Pera RR, Cullen MR. Relationship between semen production and medical comorbidity. *Fertil Steril*. 2015;103:66-71.
109. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: a cohort study of 43,277 men. *Am J Epidemiol*. 2009;170:559-565.
110. Latif T, Kold Jensen T, Mehlsen J, et al. Semen quality as a predictor of subsequent morbidity: a Danish cohort study of 4,712 men with long-term follow-up. *Am J Epidemiol*. 2017;186:910-917.
111. Peng X, Zeng X, Peng S, Deng D, Zhang J. The association risk of male subfertility and testicular cancer: a systematic review. *PLoS ONE*. 2009;4:e5591.
112. Giwercman A, Richiardi L, Kaijser M, Ekbom A, Akre O. Reduced risk of prostate cancer in men who are childless as compared to those who have fathered a child: a population based case-control study. *Int J Cancer*. 2005;115:994-997.
113. Walsh TJ, Schembri M, Turek PJ, et al. Increased risk of high-grade prostate cancer among infertile men. *Cancer*. 2010;116:2140-2147.
114. Glazer CH, Bonde JP, Giwercman A, et al. Risk of diabetes according to male factor infertility: a register-based cohort study. *Hum Reprod*. 2017;32:1474-1481.
115. Jensen TK, Andersson AM, Jorgensen N, et al. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril*. 2004;82:863-870.
116. Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Hum Reprod*. 2007;22:188-196.
117. Latif T, Lindahl-Jacobsen R, Mehlsen J, et al. Semen quality associated with subsequent hospitalizations - Can the effect be explained by socio-economic status and lifestyle factors? *Andrology*. 2018;6:428-435.
118. Andersson AM, Jorgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE. Impaired Leydig cell function in infertile men: A study of 357 idiopathic infertile men and 318 proven fertile controls. *J Clin Endocrinol Metab*. 2004;89:3161-3167.
119. Jorgensen N, Joensen UN, Toppari J, et al. Compensated reduction in Leydig cell function is associated with lower semen quality variables: a study of 8182 European young men. *Hum Reprod*. 2016;31:947-957.
120. Tajar A, Huhtaniemi IT, O'Neill TW, et al. Characteristics of androgen deficiency in late-onset hypogonadism: results from the European Male Aging Study (EMAS). *J Clin Endocrinol Metab*. 2012;97:1508-1516.
121. Andersson AM, Jensen TK, Juul A, Petersen JH, Jorgensen T, Skakkebaek NE. Secular decline in male testosterone and sex hormone binding globulin serum levels in Danish population surveys. *J Clin Endocrinol Metab*. 2007;92:4696-4705.
122. Welsh M, Suzuki H, Yamada G. The masculinization programming window. *Endocr Dev*. 2014;27:17-27.
123. Auharek SA, de Franca LR, McKinnell C, Jobling MS, Scott HM, Sharpe RM. Prenatal plus postnatal exposure to Di(n-Butyl) phthalate and/or flutamide markedly reduces final sertoli cell number in the rat. *Endocrinology*. 2010;151:2868-2875.
124. Christiansen S, Scholze M, Dalgaard M, et al. Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. *Environ Health Perspect*. 2009;117:1839-1846.
125. van den Driesche S, Kolovos P, Platts S, Drake AJ, Sharpe RM. Inter-relationship between testicular dysgenesis and Leydig cell function in the masculinization programming window in the rat. *PLoS ONE*. 2012;7:e30111.
126. Aitken RJ. Oxidative stress and the etiology of male infertility. *J Assist Reprod Genet*. 2016;33:1691-1692.
127. Aitken RJ. Reactive oxygen species as mediators of sperm capacitation and pathological damage. *Mol Reprod Dev*. 2017;84:1039-1052.
128. Bisht S, Dada R. Oxidative stress: Major executioner in disease pathology, role in sperm DNA damage and preventive strategies *Front. Biosci (Schol Ed)*. 2017;9:420-447.
129. Bui AD, Sharma R, Henkel R, Agarwal A. Reactive oxygen species impact on sperm DNA and its role in male infertility. *Andrologia*. 2018;50:e13012.
130. Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod BioMed Online*. 2014;28:684-703.
131. Agarwal A, Rana M, Qiu E, AlBunni H, Bui AD, Henkel R. Role of oxidative stress, infection and inflammation in male infertility. *Andrologia*. 2018;50:e13126.
132. Lafuente R, García-Blàque N, Jacquemin B, Checa MA. Outdoor air pollution and sperm quality. *Fertil Steril*. 2016;106:880-896.
133. Carré J, Gatimel N, Moreau J, Parinaud J, Leandri R. Does air pollution play a role in infertility?: a systematic review. *Environ Health*. 2017;16:82.
134. Zhang MH, Zhang AD, Shi ZD, Wang LG, Qiu Y. Changes in levels of seminal nitric oxide synthase, macrophage migration inhibitory factor, sperm DNA integrity and caspase-3 in fertile men after scrotal heat stress. *PLoS ONE*. 2015;10:e0141320.
135. Kunzle R, Mueller MD, Hanggi W, Birkhauser MH, Drescher H, Bersinger NA. Semen quality of male smokers and nonsmokers in infertile couples. *Fertil Steril*. 2003;79:287-291.
136. Shrivastava V, Marmor H, Chernyak S, Goldstein M, Feliciano M, Vigodner M. Cigarette smoke affects posttranslational modifications and inhibits capacitation-induced changes in human sperm proteins. *Reprod Toxicol*. 2014;43:125-129.
137. Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. *Fertil Steril*. 2011;95:116-123.
138. Bundhun PK, Janoo G, Bhurtu A, et al. Tobacco smoking and semen quality in infertile males: a systematic review and meta-analysis. *BMC Public Health*. 2019;19:36.
139. Al-Matubsi HY, Kanaan RA, Hamdan F, Salim M, Oriquat GA, Al Hanbali OA. Smoking practices in Jordanian people and their impact on semen quality and hormonal levels among adult men *Cent. Eur J Public Health*. 2011;19:54-59.
140. Anifandis G, Bounartzi T, Messini CI, Dafopoulos K, Sotiriou S, Messinis IE. The impact of cigarette smoking and alcohol consumption on sperm parameters and sperm DNA fragmentation (SDF) measured by Halosperm((R)). *Arch Gynecol Obstet*. 2014;290:777-782.
141. Caserta D, Bordi G, Di Segni N, D'Ambrosio A, Mallozzi M, Moscarini M. The influence of cigarette smoking on a population of infertile men and women. *Arch Gynecol Obstet*. 2013;287:813-818.
142. Jeng HA, Chen YL, Kantaria KN. Association of cigarette smoking with reproductive hormone levels and semen quality in healthy adult men in Taiwan. *J Environ Sci Health A Tox Hazard Subst Environ Eng*. 2014;49:262-268.
143. Lotti F, Corona G, Vitale P, et al. Current smoking is associated with lower seminal vesicles and ejaculate volume, despite higher testosterone levels, in male subjects of infertile couples. *Hum Reprod*. 2015;30:590-602.
144. Kovac JR, Khanna A, Lipshultz LI. The effects of cigarette smoking on male fertility. *Postgrad Med*. 2015;127:338-341.
145. Vanegas JC, Chavarro JE, Williams PL, et al. Discrete survival model analysis of a couple's smoking pattern and outcomes of assisted reproduction. *Fertil Res Pract* 2017; 3: pii: 5.
146. Wesselink AK, Hatch EE, Rothman KJ, Mikkelsen EM, Aschengrau A, Wise LA. Prospective study of cigarette smoking and fecundability. *Hum Reprod*. 2019;34:558-567.
147. ASRM (Practice Committee of the American Society for Reproductive Medicine). Smoking and infertility: a committee opinion. *Fertil Steril*. 2018;110:611-618.

148. El Golli N, Rahali D, Jrad-Lamine A, et al. Impact of electronic-cigarette refill liquid on rat testis *Toxicol Mech. Methods*. 2016;26:427-434.
149. Rahali D, Jrad-Lamine A, Dallagi Y, et al. Semen parameter alteration, histological changes and role of oxidative stress in adult rat epididymis on exposure to electronic cigarette refill liquid. *Chin J Physiol*. 2018;61:75-84.
150. Hammond D. Outbreak of pulmonary diseases linked to vaping. *BMJ*. 2019;366:l5445.
151. Ricci E, Vigano P, Cipriani S, et al. Coffee and caffeine intake and male infertility: a systematic review. *Nutr J*. 2017;16:37.
152. Jensen TK, Gottschau M, Madsen JO, et al. Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *BMJ Open*. 2014;4:e005462.
153. Muthusami KR, Chinnaswamy P. Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertil Steril*. 2005;84:919-924.
154. Ricci E, Al Beitawi S, Cipriani S, et al. Semen quality and alcohol intake: a systematic review and meta-analysis. *Reprod Biomed Online*. 2017;34:38-47.
155. Nargund VH. Effects of psychological stress on male fertility. *Nat Rev Urol*. 2015;12:373-382.
156. Gollenberg AL, Liu F, Brazil C, et al. Semen quality in fertile men in relation to psychosocial stress. *Fertil Steril*. 2010;93:1104-1111.
157. Nordkap L, Jensen TK, Hansen AM, et al. Psychological stress and testicular function: a cross-sectional study of 1,215 Danish men. *Fertil Steril*. 2016;105:174-187.
158. Arya ST, Dibb B. The experience of infertility treatment: the male perspective. *Hum Fertil (Camb)*. 2016;19:242-248.
159. Drobnis EZ, Nangia AK. Psychotropics and male reproduction. *Adv Exp Med Biol*. 2017;1034:63-101.
160. Frederiksen Y, Farver-Vestergaard I, Skovgard NG, Ingerslev HJ, Zachariae R. Efficacy of psychosocial interventions for psychological and pregnancy outcomes in infertile women and men: a systematic review and meta-analysis. *BMJ Open*. 2015;5:e006592.
161. Palnitkar G, Phillips CL, Hoyos CM, Marren AJ, Bowman MC, Yee BJ. Linking sleep disturbance to idiopathic male infertility. *Sleep Med Rev*. 2018;42:149-159.
162. Jensen TK, Andersson AM, Skakkebaek NE, et al. Association of sleep disturbances with reduced semen quality: a cross-sectional study among 953 healthy young Danish men. *Am J Epidemiol*. 2013;177:1027-1037.
163. Jensen TK, Heitmann BL, Blomberg Jensen M, et al. High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. *Am J Clin Nutr*. 2013;97:411-418.
164. Luboshitzky R, Zabari Z, Shen-Orr Z, Herer P, Lavie P. Disruption of the nocturnal testosterone rhythm by sleep fragmentation in normal men. *J Clin Endocrinol Metab*. 2001;86:1134-1139.
165. Zhang W, Piotrowska K, Chavoshan B, Wallace J, Liu PY. Sleep duration is associated with testis size in healthy young men. *J Clin Sleep Med*. 2018;14:1757-1764.
166. Eisele HJ, Markart P, Schulz R. Obstructive sleep apnea, oxidative stress, and cardiovascular disease: evidence from human studies. *Oxid Med Cell Longev*. 2015;2015:608438.
167. Janevic T, Kahn LG, Landsbergis P, et al. Effects of work and life stress on semen quality. *Fertil Steril*. 2014;102:530-538.
168. Radwan M, Jurewicz J, Merecz-Kot D, et al. Sperm DNA damage-the effect of stress and everyday life factors. *Int J Impot Res*. 2016;28:148-154.
169. Wdowiak A, Bien A, Iwanowicz-Palus G, Makara-Studzinska M, Bojar I. Impact of emotional disorders on semen quality in men treated for infertility. *Neuro Endocrinol Lett*. 2017;38:50-58.
170. Salas-Huetos A, Bullo M, Salas-Salvado J. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. *Hum Reprod Update*. 2017;23:371-389.
171. Afeiche M, Williams PL, Mendiola J, et al. Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men. *Hum Reprod*. 2013;28:2265-2275.
172. Sermondade N, Faure C, Fezeu L, et al. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Hum Reprod Update*. 2013;19:221-231.
173. Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Buck Louis GM. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Hum Reprod*. 2014;29:193-200.
174. Campbell JM, Lane M, Owens JA, Bakos HW. Paternal obesity negatively affects male fertility and assisted reproduction outcomes: a systematic review and meta-analysis. *Reprod Biomed Online*. 2015;31:593-604.
175. Bieniek JM, Kashanian JA, Deibert CM, et al. Influence of increasing body mass index on semen and reproductive hormonal parameters in a multi-institutional cohort of subfertile men. *Fertil Steril*. 2016;106:1070-1075.
176. El Bardisi H, Majzoub A, Arafa M, et al. Effect of bariatric surgery on semen parameters and sex hormone concentrations: a prospective study. *Reprod Biomed Online*. 2016;33:606-611.
177. Faure C, Dupont C, Baraibar MA, et al. In subfertile couple, abdominal fat loss in men is associated with improvement of sperm quality and pregnancy: a case-series. *PLoS ONE*. 2014;9:e86300.
178. Hakonsen LB, Thulstrup AM, Aggerholm AS, et al. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health*. 2011;8:24.
179. Kahn BE, Brannigan RE. Obesity and male infertility. *Curr Opin Urol*. 2017;27:441-445.
180. Samavat J, Cantini G, Lotti F, et al. Massive weight loss obtained by bariatric surgery affects semen quality in morbid male obesity: a preliminary prospective double-armed study. *Obes Surg*. 2018;28:69-76.
181. Legro RS, Kunselman AR, Meadows JW, et al. Time-related increase in urinary testosterone levels and stable semen analysis parameters after bariatric surgery in men. *Reprod Biomed Online*. 2015;30:150-156.
182. Lazaros L, Hatzi E, Markoula S, et al. Dramatic reduction in sperm parameters following bariatric surgery: report of two cases. *Andrologia*. 2012;44:428-432.
183. Sermondade N, Massin N, Boitrelle F, et al. Sperm parameters and male fertility after bariatric surgery: three case series. *Reprod Biomed Online*. 2012;24:206-210.
184. Rodprasert W, Main KM, Toppari J, Virtanen HE. Associations between male reproductive health and exposure to endocrine disrupting chemicals. *Curr Opin Endocrine Metabolic Res*. 2019; 7: 49-61.
185. Bergman A, Heindel J, Jobling S, Kidd K, Zoeller R. *WHO-UNEP (World Health Organisation and United Nations Environment Program) report on State of the Science of Endocrine Disrupting Chemicals*. Geneva: WHO Press; 2012.
186. EEA (European Environment Agency)The impacts of endocrine disrupters on wildlife, people and their environments The Weybridge+15 (1996–2011) report. Publications Office of the European Union; 2012. ISBN 978-92-9213-307-8, ISSN 1725-2237; https://doi.org/10.2800/41462.
187. Kabir ER, Rahman MS, Rahman I. A review on endocrine disruptors and their possible impacts on human health. *Environ Toxicol Pharmacol*. 2015;40:241-258.
188. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: The endocrine society's second scientific statement on endocrine-disrupting chemicals. *Endocr Rev*. 2015;36:E1-E150.

189. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet*. 1993;341:1392-1395.
190. Juul A, Almstrup K, Andersson AM, et al. Possible fetal determinants of male infertility. *Nat Rev Endocrinol*. 2014;10:553-562.
191. Bliatka D, Lymperi S, Mastorakos G, Goulis DG. Effect of endocrine disruptors on male reproduction in humans: why the evidence is still lacking? *Andrology*. 2017;5:404-407.
192. Wang C, Yang L, Wang S, et al. The classic EDCs, phthalate esters and organochlorines, in relation to abnormal sperm quality: a systematic review with meta-analysis. *Sci Rep*. 2016;6:19982.
193. Bonde JP, Flachs EM, Rimborg S, et al. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. *Hum Reprod Update*. 2016;23:104-125.
194. Di Nisio A, Foresta C. Water and soil pollution as determinant of water and food quality/contamination and its impact on male fertility. *Reprod Biol Endocrinol*. 2019;17:4.
195. Conley JM, Lambright CS, Evans N, et al. Mixed "Antiandrogenic" chemicals at low individual doses produce reproductive tract malformations in the male rat. *Toxicol Sci*. 2018;164:166-178.
196. Kanayama G, Pope HG Jr. History and epidemiology of anabolic androgens in athletes and non-athletes. *Mol Cell Endocrinol*. 2018;464:4-13.
197. Duca Y, Aversa A, Condorelli RA, Calogero AE, La Vignera S. Substance abuse and male hypogonadism. *J Clin Med*. 2019;8:E732.
198. Sagoe D, Molde H, Andreassen CS, Torsheim T, Pallesen S. The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. *Ann Epidemiol*. 2014;24:383-398.
199. El Osta R, Almont T, Diligent C, Hubert N, Eschwège P, Hubert J. Anabolic steroids abuse and male infertility. *Basic Clin Androl*. 2016;26:2.
200. Ip EJ, Barnett MJ, Tenerowicz MJ, Perry PJ. The Anabolic 500 survey: characteristics of male users versus nonusers of anabolic-androgenic steroids for strength training. *Pharmacotherapy*. 2011;31:757-766.
201. Kanayama G, Pope HG Jr. Illicit use of androgens and other hormones: recent advances. *Curr Opin Endocrinol Diabetes Obes*. 2012;19:211-219.
202. Mowat A, Newton C, Boothroyd C, Demmers K, Fleming S. The effects of vaginal lubricants on sperm function: an in vitro analysis. *J Assist Reprod Genet*. 2014;31:333-339.
203. Sandhu RS, Wong TH, Kling CA, Chohan KR. vitro effects of coital lubricants and synthetic and natural oils on sperm motility. *Fertil Steril*. 2014;101:941-944.
204. Wilson SL, Adam JK, Krishna SBN. Effects of vaginal lubricants on in-vitro progressive spermatozoa motility. *Afr J Reprod Health*. 2017;21:96-101.
205. Steiner AZ, Long DL, Tanner C, Herring AH. Effect of vaginal lubricants on natural fertility. *Obstet Gynecol*. 2012;120:44-51.
206. Mackenzie SC, Gellatly SA. Vaginal lubricants in the couple trying-to-conceive: Assessing healthcare professional recommendations and effect on in vitro sperm function. *PLoS ONE*. 2019;14(5):e0209950.
207. Mortimer D, Barratt CL, Björndahl L, de Jager C, Jequier AM, Muller CH. What should it take to describe a substance or product as 'sperm-safe'. *Hum Reprod Update*. 2013;19(Suppl 1):i1-45.
208. NICE. National Institute for Health and Clinical Excellence: Guidance in *Fertility: Assessment and Treatment for People with Fertility Problems*; 2013. https://www.nice.org.uk/guidance/cg156.
209. Whorton D, Foliart D. DBCP: eleven years later. *Reprod Toxicol*. 1988;2:155-161.
210. Whorton D, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. *Lancet*. 1977;2:1259-1261.
211. Wyrobek AJ. Methods for evaluating the effects of environmental chemicals on human sperm production. *Environ Health Perspect*. 1983;48:53-59.
212. Schrader SM, Marlow KL. Assessing the reproductive health of men with occupational exposures. *Asian J Androl*. 2014;16:23-30.
213. Cherry N, Moore H, McNamee R, et al. Occupation and male infertility: glycol ethers and other exposures. *Occup Environ Med*. 2008;65:708-714.
214. Cherry N, Povey AC, McNamee R, et al. Occupation exposures and sperm morphology: a case-referent analysis of a multi-centre study. *Occup Environ Med*. 2014;71:598-604.
215. Povey AC, Clyma JA, McNamee R, et al. Modifiable and non-modifiable risk factors for poor semen quality: a case-referent study. *Hum Reprod*. 2012;27:2799-2806.
216. Pacey AA, Povey AC, Clyma JA, et al. Modifiable and non-modifiable risk factors for poor sperm morphology. *Hum Reprod*. 2014;29:1629-1636.
217. Snijder CA, te Velde E, Roeleveld N, Burdorf A. Occupational exposure to chemical substances and time to pregnancy: a systematic review. *Hum Reprod Update*. 2012;18:284-300.
218. Bonde JP. Occupational risk for male infertility. *Occup Environ Med*. 2014;71:596-597.
219. Krausz C, Riera-Escamilla A. Genetics of male infertility. *Nat Rev Urol*. 2018;15:369-384.
220. Krausz C, Cioppi F, Riera-Escamilla A. Testing for genetic contributions to infertility: potential clinical impact. *Expert Rev Mol Diagn*. 2018;18:331-346.
221. Punab M, Poolamets O, Paju P, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. *Hum Reprod*. 2017;32:18-31.
222. Tournaye H, Krausz C, Oates RD. Concepts in diagnosis and therapy for male reproductive impairment. *Lancet Diabetes Endocrinol*. 2017;5:554-564.
223. Tournaye H, Krausz C, Oates RD. Novel concepts in the aetiology of male reproductive impairment. *Lancet Diabetes Endocrinol*. 2017;5:544-553.
224. Krausz C, Hoefsloot L, Simoni M, Tuttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. *Andrology*. 2014;2:5-19.
225. Krausz C, Escamilla AR, Chianese C. Genetics of male infertility: from research to clinic. *Reproduction*. 2015;150:R159-174.
226. Ropke A, Tuttelmann F. Mwechanisms of endocrinology: Aberrations of the X chromosome as cause of male infertility. *Eur J Endocrinol*. 2017;177:R249-R259.
227. Coutton C, Escoffier J, Martinez G, Arnoult C, Ray PF. Teratozoospermia: spotlight on the main genetic actors in the human. *Hum Reprod Update*. 2015;21:455-485.
228. Jenkins TG, Aston KI, James ER, Carrell DT. Sperm epigenetics in the study of male fertility, offspring health, and potential clinical applications. *Syst Biol Reprod Med*. 2017;63:69-76.
229. Klaver R, Gromoll J. Bringing epigenetics into the diagnostics of the andrology laboratory: challenges and perspectives. *Asian J Androl*. 2014;16:669-674.
230. Kaneshiro KR, Rechtsteiner A, Strome S. Sperm-inherited H3K27me3 impacts offspring transcription and development in C elegans. *Nat Commun*. 2019;10:1271.
231. Monk D, Mackay DJG, Eggermann T, Maher ER, Riccio A. Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. *Nat Rev Genet*. 2019;20:235-248.
232. Krausz C, Riera-Escamilla A, Chianese C, et al. From exome analysis in idiopathic azoospermia to the identification of a high-risk subgroup for occult Fanconi anemia. *Genet Med*. 2019;21:189-194.

233. Krausz C. Male infertility: pathogenesis and clinical diagnosis. *Best Pract Res Clin Endocrinol Metab*. 2011;25:271-285.
234. Khourdaji I, Lee H, Smith RP. Frontiers in hormone therapy for male infertility. *Transl Androl Urol*. 2018;7:S353-S366.
235. Farhat R, Al-zidjali F, Alzahrani AS. Outcome of gonadotropin therapy for male infertility due to hypogonadotrophic hypogonadism. *Pituitary*. 2010;13:105-110.
236. Liu PY, Baker HW, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. *J Clin Endocrinol Metab*. 2009;94:801-808.
237. Delemarre-Van de Waal HA, Odink RJ. Pulsatile GnRH treatment in boys and girls with idiopathic hypogonadotrophic hypogonadism. *Hum Reprod* 1993;8(Suppl 2):180-183.
238. Pitteloud N, Acierno JS Jr, Meysing AU, Dwyer AA, Hayes FJ, Crowley WF Jr. Reversible kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab* 2005; 90: 1317-1322.
239. Sykiotis GP, Hoang XH, Avbelj M, et al. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. *J Clin Endocrinol Metab* 2010; 95: 3019-3027.
240. Mao JF, Liu ZX, Nie M, et al. Pulsatile gonadotropin-releasing hormone therapy is associated with earlier spermatogenesis compared to combined gonadotropin therapy in patients with congenital hypogonadotropic hypogonadism. *Asian J Androl*. 2017;19:680-685.
241. Cailleux-Bounacer A, Reznik Y, Cauliez B, Menard JF, Duparc C, Kuhn JM. Evaluation of endocrine testing of Leydig cell function using extractive and recombinant human chorionic gonadotropin and different doses of recombinant human LH in normal men. *Eur J Endocrinol*. 2008;159:171-178.
242. Buchter D, Behre HM, Kliesch S, Nieschlag E. Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. *Eur J Endocrinol*. 1998;139:298-303.
243. Ishikawa T, Ooba T, Kondo Y, Yamaguchi K, Fujisawa M. Assessment of gonadotropin therapy in male hypogonadotropic hypogonadism. *Fertil Steril*. 2007;88:1697-1699.
244. Dwyer AA, Sykiotis GP, Hayes FJ, et al. Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2013;98:E1790-1795.
245. Majumdar SS, Sarda K, Bhattacharya I, Plant TM. Insufficient androgen and FSH signaling may be responsible for the azoospermia of the infantile primate testes despite exposure to an adult-like hormonal milieu. *Hum Reprod*. 2012;27:2515-2525.
246. Rastrelli G, Corona G, Mannucci E, Maggi M. Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. *Andrology*. 2014;2:794-808.
247. Nieschlag E, Bouloux PG, Stegmann BJ, et al. An open-label clinical trial to investigate the efficacy and safety of corifollitropin alfa combined with hCG in adult men with hypogonadotropic hypogonadism. *Reprod Biol Endocrinol*. 2017;15:17.
248. Mao JF, Xu HL, Duan J, et al. Reversal of idiopathic hypogonadotropic hypogonadism: a cohort study in Chinese patients. *Asian J Androl*. 2015;17:497-502.
249. Practice Committee of the American Society for Reproductive Medicine Electronic Address. Management of nonobstructive azoospermia: a committee opinion. *Fertil Steril* 2018; 110: 1239-1245.
250. Colpi GM, Francavilla S, Haidl G, et al. European academy of andrology guideline management of oligo-astheno-teratozoospermia. *Andrology*. 2018;6:513-524.
251. Jung JH, Seo JT. Empirical medical therapy in idiopathic male infertility: Promise or panacea? *Clin Exp Reprod Med*. 2014;41:108-114.
252. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev*. 2014;CD007411.
253. Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. *Cochrane Database Syst Rev*. 2019;3:CD007411.
254. Calogero AE, Condorelli RA, Russo GI, La Vignera S. Conservative nonhormonal options for the treatment of male infertility: antibiotics, anti-inflammatory drugs, and antioxidants. *Biomed Res Int*. 2017;2017:4650182.
255. Barratt CLR. A Dolce & Gabbana model in every ART clinic? *Mol Hum Reprod*. 2018;24:431-432.
256. Salas-Huetos A, Rosique-Esteban N, Becerra-Tomas N, Vizmanos B, Bullo M, Salas-Salvado J. The effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and meta-analysis of randomized clinical trials. *Adv Nutr*. 2018;9:833-848.
257. Porat-Soldin O, Soldin SJ. Preliminary studies on the in vitro and in vivo effect of salicylate on sperm motility. *Ther Drug Monit*. 1992;14:366-370.
258. Roodbari F, Abedi N, Talebi AR. Early and late effects of Ibuprofen on mouse sperm parameters, chromatin condensation, and DNA integrity in mice *Iran. J Reprod Med*. 2015;13:703-710.
259. Vargas A, Bustos-Obregon E, Hartley R. Effects of hypoxia on epididymal sperm parameters and protective role of ibuprofen and melatonin. *Biol Res*. 2011;44:161-167.
260. Canale D, Scaricabarozzi I, Giorgi P, Turchi P, Ducci M, Menchini-Fabris GF. Use of a novel non-steroidal anti-inflammatory drug, nimesulide, in the treatment of abacterial prostatovesiculitis. *Andrologia*. 1993;25:163-166.
261. Gambera L, Serafini F, Morgante G, Focarelli R, De Leo V, Piomboni P. Sperm quality and pregnancy rate after COX-2 inhibitor therapy of infertile males with abacterial leukocytospermia. *Hum Reprod*. 2007;22:1047-1051.
262. Bals-Pratsch M, Doren M, Karbowski B, Schneider HP, Nieschlag E. Cyclic corticosteroid immunosuppression is unsuccessful in the treatment of sperm antibody-related male infertility: a controlled study. *Hum Reprod*. 1992;7:99-104.
263. Haas GG Jr, Manganiello P. A double-blind, placebo-controlled study of the use of methylprednisolone in infertile men with sperm-associated immunoglobulins. *Fertil Steril*. 1987;47:295-301.
264. Lahteenmaki A, Rasanen M, Hovatta O. Low-dose prednisolone does not improve the outcome of in-vitro fertilization in male immunological infertility. *Hum Reprod*. 1995;10:3124-3129.
265. Hendry WF, Hughes L, Scammell G, Pryor JP, Hargreave TB. Comparison of prednisolone and placebo in subfertile men with antibodies to spermatozoa. *Lancet*. 1990;335:85-88.
266. Omu AE, al-QattanF, Abdul HamadaB. Effect of low dose continuous corticosteroid therapy in men with antisperm antibodies on spermatozoal quality and conception rate. *Eur J Obstet Gynecol Reprod Biol*. 1996;69:129-134.
267. Taiyeb AM, Ridha-Albarzanchi MT, Taiyeb SM, et al. Improvement in pregnancy outcomes in couples with immunologically male infertility undergoing prednisolone treatment and conventional in vitro fertilization preceded by sperm penetration assay: a randomized controlled trial. *Endocrine*. 2017;58:448-457.
268. Jung JH, Kim MH, Kim J, et al. Treatment of leukocytospermia in male infertility: a systematic review. *World J Mens Health*. 2016;34:165-172.
269. Baker HW, Straffon WG, McGowan MP, Burger HG, de Kretser DM, Hudson B. A controlled trial of the use of erythromycin for men with asthenospermia. *Int J Androl*. 1984;7:383-388.

270. Comhaire FH, Rowe PJ, Farley TM. The effect of doxycycline in infertile couples with male accessory gland infection: a double blind prospective study. *Int J Androl*. 1986;9:91-98.
271. Vicari E. Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. *Hum Reprod*. 2000;15:2536-2544.
272. Erel CT, Senturk LM, Demir F, Irez T, Ertungealp E. Antibiotic therapy in men with leukocytospermia. *Int J Fertil Womens Med*. 1997;42:206-210.
273. Krisp A, Horster S, Skrzypek J, Krause W. Treatment with levofloxacin does not resolve asymptomatic leucocytospermia–a randomized controlled study. *Andrologia*. 2003;35:244-247.
274. Yanushpolsky EH, Politch JA, Hill JA, Anderson DJ. Antibiotic therapy and leukocytospermia: a prospective, randomized, controlled study. *Fertil Steril*. 1995;63:142-147.
275. Lewis-Jones DI, Kynaston HG, Lynch RV, Desmond AD, Entwhistle PA. Antibiotic treatment of leucospermia in subfertile males. *Arch STD/HIV Res*. 1996;10:65-72.
276. Branigan EF, Muller CH. Efficacy of treatment and recurrence rate of leukocytospermia in infertile men with prostatitis. *Fertil Steril*. 1994;62:580-584.
277. Yamamoto M, Hibi H, Katsuno S, Miyake K. Antibiotic and ejaculation treatments improve resolution rate of leukocytospermia in infertile men with prostatitis. *Nagoya J Med Sci*. 1995;58:41-445.
278. Drobnis EZ, Nangia AK. Antimicrobials and male reproduction. *Adv Exp Med Biol*. 2017;1034:131-161.
279. Khaki A. Assessment on the adverse effects of Aminoglycosides and Flouroquinolone on sperm parameters and male reproductive tissue: A systematic review *Iran*. *J Reprod Med*. 2015;13:125-134.
280. Samplaski MK, Nangia AK. Adverse effects of common medications on male fertility. *Nat Rev Urol*. 2015;12:401-413.
281. Boulet SL, Mehta A, Kissin DM, Warner L, Kawwass JF, Jamieson DJ. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA*. 2015;313:255-263.
282. Evers JL. Santa Claus in the fertility clinic. *Hum Reprod*. 2016;31:1381-1382.
283. Dyer S, Chambers GM, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. *Hum Reprod*. 2016;31:1588-1609.
284. CDC (Centers for Disease Control and Prevention) American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2016 Assisted Reproductive Technology National Summary Report. Atlanta (GA): US Dept of Health and Human Services; 2018.
285. Mortimer D, Mortimer ST. The case against intracytoplasmic sperm injection for all. In: Aitken RJ, Mortimer D, Kovacs GT, eds. *Male and sperm factors that maximize IVF success*. Cambridge: Cambridge University Press; 2020.
286. Li Z, Wang AY, Bowman M, et al. ICSI does not increase the cumulative live birth rate in non-male factor infertility. *Hum Reprod*. 2018;33:1322-1330.
287. Sustar K, Rozen G, Agresta F, Polyakov A. Use of intracytoplasmic sperm injection (ICSI) in normospermic men may result in lower clinical pregnancy and live birth rates. *Aust N Z J Obstet Gynaecol*. 2019;59:706-711.
288. Miller D, Pavitt S, Sharma V, et al. Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. *Lancet*. 2019;393:416-422.
289. Berntsen S, Söderström-Anttila V, Wennerholm UB, et al. The health of children conceived by ART: 'the chicken or the egg?' *Hum Reprod Update*. 2019;25:137-158.
290. Catford SR, McLachlan RI, O'Bryan MK, Halliday JL. Long-term follow-up of intra-cytoplasmic sperm injection-conceived offspring compared with in vitro fertilization-conceived offspring: a systematic review of health outcomes beyond the neonatal period. *Andrology*. 2017;5:610-621.
291. Rumbold AR, Sevoyan A, Oswald TK, Fernandez RC, Davies MJ, Moore VM. Impact of male factor infertility on offspring health and development. *Fertil Steril*. 2019;111:1047-1053.
292. Alley RB, Emanuel KA, Zhang F. Advances in weather prediction. *Science*. 2019;363:342-344.

How to cite this article: Cairo Consensus Workshop Group. The current status and future of andrology: A consensus report from the Cairo workshop group. *Andrology*. 2019;0:1–26. https://doi.org/10.1111/andr.12720