Male Infertility: New Developments, Current Challenges, and Future Directions

Murat Gül, Giorgio Ivan Russo, Hussein Kandil, Florence Boitrelle, Ramadan Saleh, Eric Chung, Parviz Kavoussi, Taymour Mostafa, Rupin Shah, Ashok Agarwal

Review Article
Male reproductive health and infertility

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health Published online Jan 2, 2024
https://doi.org/10.5534/wjmh.230232

The World Journal of MEN's HEALTH

Male Infertility: New Developments, Current Challenges, and Future Directions

Murat Gül[1,13], Giorgio Ivan Russo[2,13], Hussein Kandil[3,13], Florence Boitrelle[4,5,13], Ramadan Saleh[6,7,13], Eric Chung[8,13], Parviz Kavoussi[9,13], Taymour Mostafa[10,13], Rupin Shah[11,12,13], Ashok Agarwal[13,14]

[1]Department of Urology, Selcuk University School of Medicine, Konya, Turkey, [2]Urology Section, University of Catania, Catania, Italy, [3]Fakih IVF Fertility Center, Abu Dhabi, UAE, [4]Reproductive Biology, Fertility Preservation, Andrology, CECOS, Poissy Hospital, Poissy, France, [5]Paris Saclay University, UVSQ, INRAE, BREED, Jouy-en-Josas, France, [6]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag University, Sohag, [7]Ajyal IVF Center, Ajyal Hospital, Sohag, Egypt, [8]Department of Urology, Princess Alexandra Hospital, University of Queensland, Brisbane, QLD, Australia, [9]Department of Reproductive Urology, Austin Fertility & Reproductive Medicine/ Westlake IVF, Austin, TX, USA, [10]Department of Andrology, Sexology and STIs, Faculty of Medicine, Cairo University, Cairo, Egypt, [11]Department of Urology, Lilavati Hospital and Research Centre, Mumbai, [12]Well Women's Centre, Sir HN Reliance Foundation Hospital, Mumbai, India, [13]Global Andrology Forum, Moreland Hills, OH, [14]Cleveland Clinic, Cleveland, OH, USA

There have been many significant scientific advances in the diagnostics and treatment modalities in the field of male infertility in recent decades. Examples of these include assisted reproductive technologies, sperm selection techniques for intracytoplasmic sperm injection, surgical procedures for sperm retrieval, and novel tests of sperm function. However, there is certainly a need for new developments in this field. In this review, we discuss advances in the management of male infertility, such as seminal oxidative stress testing, sperm DNA fragmentation testing, genetic and epigenetic tests, genetic manipulations, artificial intelligence, personalized medicine, and telemedicine. The role of the reproductive urologist will continue to expand in future years to address different topzics related to diverse questions and controversies of pathophysiology, diagnosis, and therapy of male infertility, training researchers and physicians in medical and scientific research in reproductive urology/ andrology, and further development of andrology as an independent specialty.

Keywords: DNA fragmentation; Epigenomics; Infertility, male; Spermatozoa

INTRODUCTION

Infertility is a common medical problem, with the male factor being involved in approximately 50% of cases [1,2]. Male infertility has been linked to numerous genetic and lifestyle factors, but approximately 30% of cases are still recognized as idiopathic [3]. Over the last 40 years, developments in male infertility diagnostics, such as genomics and molecular testing, have helped elucidate etiologies for what was previously considered unexplained infertility. However, there is a need for new research to fill the gaps in knowledge on the etiologies of male infertility and to offer definite solutions for many of these cases that are still untreatable. This review aims to identify areas of deficiency in the knowledge, discuss new diagnostic methods and thera-

Received: Aug 16, 2023 **Accepted:** Aug 27, 2023 **Published online** Jan 2, 2024
Correspondence to: Ashok Agarwal https://orcid.org/0000-0003-0585-1026
Global Andrology Forum, 130 West Juniper Lane, Moreland Hills, OH 44022, USA.
Tel: +1-216-312-5829, **E-mail:** agarwa32099@outlook.com, **Website:** https://www.globalandrologyforum.com

pies that are being developed as well as potential targets for new therapies, and provide insights to identify future areas for both research and therapy in male infertility.

TOWARDS A BETTER UNDERSTANDING OF THE ETIOPATHOGENESIS OF MALE INFERTILITY

Different factors are implicated in spermatogenic aberration and consequently male infertility, including environmental, genetic, inflammatory, infective, drug-induced, and hormonal disorders, in addition to anatomic etiologies such as varicoceles and reproductive tract obstructions [4]. Varicocele is considered the most common correctable cause of male infertility with an incidence of approximately 35% among men with primary infertility and 70% to 80% among men with secondary infertility [5]. Currently, many studies indicate a correlation between varicocele and the progressive decline in testicular function. Hypotheses for the induction of spermatogenic dysfunction induced by varicocele include elevated intra-scrotal temparature, oxidative stress (OS), seminiferous tubules hypoxia, venous reflux, backflow of adrenal metabolites, and sperm DNA fragmentation (SDF) [6]. A recent systematic review and meta-analysis supported the relationship between varicocele repair and improved semen parameters [7], while a study conducted by Panach-Navarrete et al [8] concluded that varicocele repair improves semen analysis (SA) parameters only in patients with decreased baseline parameters. Further, a meta-analysis from Chen et al [9] reported increased testosterone levels in hypogonadal males who underwent varicocele repair but there is still controversy about the impact of the grades of varicocele, size of testes, presence of other medical comorbidities or duration of varicocele on testosterone production.

An increase in endocrine-mediated environmental and lifestyle factors having a detrimental impact on male fertility has been reported [10]. A significantly lower total sperm count has been reported among e-cigarette and cigarette smokers [11]. A study by Holmboe et al [12] revealed that compared to non-smokers, both daily e-cigarette users, and daily cigarette smokers had significantly lower total sperm counts (147 million *vs.* 91 million and 139 million *vs.* 103 million respectively) in an adjusted analysis. However, higher levels of total and free testosterone were observed in cigarette users only and no association was observed in e-cigarette smokers [12]. Although clinically it is well accepted that smoking has an adverse impact on spermatozoa, more research on the impact of smoking and e-cigarettes and also of passive smoking on male fertility is warranted. Similarly, several population-based surveys reported an increasing percentage of altered semen parameters among overweight males [13]. Few mechanisms that alter reproductive function among obese males have been reported, including increased serum estradiol levels and a higher level of serum leptin, which directly results in testosterone downregulation, erectile dysfunction, and higher inflammatory mediators [14,15]. This data has brought attention to the consideration of nutrigenomics in male infertility where interaction between nutrients, diet, and various genes expression may play an important role in health and development. In rats, a high-fat diet has been shown to influence pre-implantation embryo gene expression, fetal growth of the offspring, and the metabolism of the adult [16]. In fact, according to a recent study by Cannarella et al [17], male obesity is related to a mutated sperm DNA methylation sequence that seems to involve reprogramming fidelity in a set of genes. Another recent study suggests that overweight boys are more likely to be infertile men. This study revealed that obese pre-pubertal boys had smaller testicles than normal pre-pubertal boys who were not obese [17].

Together with the lifestyle impact on male factor fertility, there is a growing concern about the global reduction of semen quality due to environmental pollution influenced by different endocrine-disrupting factors affecting male reproductive system [18,19]. Environmental factors can also cause epigenomic changes through DNA methylation, histone modifications, and non-coding (ncRNAs) that play a key role in the proper functioning of cells, including spermatozoa [20].

Lately, data emerging from the COVID-19 pandemic indicate that male patients account for 56% to 73% of the infected population [21-23]. SARS-CoV-2-infected males were found to have higher morbidity and mortality rates than age-matched females, suggesting sex-based differences in the prevalence and severity of COVID-19 [24]. For the SARS-CoV-2 virus, the mechanism of cellular entry has been identified as the interaction between the SARS-CoV-2 viral spike protein

and angiotensin-converting enzyme 2 (ACE2) on cells co-expressing ACE2 and the cellular transmembrane protease serine-2 [25,26]. The testis has high levels of ACE2 expression [27]. The ACE2 converts Angiotensin II to Angiotensin 1–7 in Leydig cells and adjusts the production of testosterone and consequently may contribute to spermatogenesis modulation, which suggests a potential for the negative influence of the virus on male fertility [28]. A Belgian study suggests that even men who contract COVID-19 with very mild illness and are afebrile may have an impact on their semen parameters and SDF for 3 to 6 months due to an inflammatory response [29]. In a small number of fatal cases of COVID-19, pathology at the time of autopsy has demonstrated orchitis, basal membrane thickening, vascular changes, scarcity of Leydig cells and Sertoli cells, and reduced spermatogenesis. Additional research is needed to detect the long-term impact of COVID-19 infection on male reproductive health, especially in cases that were not severe.

Existing evidence suggest that OS may play a role in male infertility. The term Male Oxidative Stress Infertility (MOSI) has been suggested for diagnosing a subset of infertile males with abnormal semen parameters, previously described as idiopathic [30]. So far, although the measurement of OS in semen is not used routinely, the introduction of novel technologies that promptly detect seminal OS *via* assessment of oxidation-reduction potential (ORP) using a bench-top analyzer permits an accurate and cost-effective diagnosis of MOSI [31]. Varicoceles are known to induce OS and varicocele repair has been demonstrated to reduce OS [32].

MALE INFERTILITY DIAGNOSTICS

1. The 6th edition of the WHO manual for semen analysis

The recommendations for the initial assessment of the infertile couple have been recently updated [33]. Along with a thorough history and physical examination, it is recommended that a SA be ordered. Since 1980, the WHO has attempted to standardize the methodology of semen examination through six editions of laboratory manuals to examine human semen with the latest (6th) edition being published in July 2021 [34].

The main novelty in the 6th edition lies in the absence of recommended SA reference values. In the 6th edition, the 5th percentile values of basic semen parameters are provided and vary very slightly from the 5th edition. These 5th percentile values were established by analyzing the semen parameters of 3,989 males who were able to initiate a natural pregnancy with a time to pregnancy of less than 12 months [1]. However, the 6th edition specifies that these 5th percentile values are only one way to assess the male fertility potential [1,34]. Certainly, semen parameters and their thresholds alone are not sufficient to predict the couples' fertility potential due to the complex and multifactorial nature of fertility.

Testing for SDF is indicated to complete the diagnostic evaluation in specific circumstances. SDF testing has been described as an "extended examination" of semen in the 6th edition of the WHO Manual of Human Semen Analysis [1]. However, the 6th edition neither provides indications for SDF testing nor does it address the variability of test results with different assays. Therefore, clinicians need to rely on the recommendations available in recent systematic reviews, meta-analyses, and guidelines regarding the causes of SDF, the indications for SDF testing, and possible treatments in patients with high SDF levels [35-39].

In the research section (advanced SA), the 6th edition of the WHO manual lists some tests to assess seminal OS (1). Despite a growing number of publications, international societies have not yet taken up this subject and the role of OS on male fertility is still not given due importance, perhaps because testing of OS is variable and may lack standardization. Well-designed studies will undoubtedly improve the utility of these tests and define their usefulness in managing male infertility in the future.

Finally, the new 6th edition of the WHO manual of SA does not detail all the new tests available for genetic and epigenetic diagnosis. This is likely due to the uncertainty of the indications and clinical utility of these tests currently. Given the cost and the complexity of implementation and clinical interpretation of these genetic and epigenetic tests, recommendations are difficult to be established from the literature. While recent guidelines recommend karyotyping and Y-chromosome microdeletions in the work-up of non-obstructive causes of severe oligozoospermia and azoospermia [33], the clinical indications for whole exome or genome studies, seminal microRNA, DNA methylation, or histone post-translational modification tests have not yet been determined. Regarding genetic test-

ing alone, literature reviews are regularly published, and a multiple number of genes have been described in male infertility [40,41]. However, it seems premature to recommend these genomic or pan-genomic analyses to the "general" infertile population. Their routine screening is not yet applicable in andrology and assisted reproductive technology (ART) centers around the world, but with further advances in the field, these tests may become routine in the evaluation of infertile males in future.

The strength of the WHO 6th edition manual is in its excellence as a technical guide. It is important that the technical recommendations are actually followed by andrology laboratories around the world to ensure the quality, consistency, and reproducibility of testing from one laboratory to another. Clinically, what is considered a weakness of the manual is its lack of criteria for the clinical interpretation of these tests. It is clear that the literature does not allow the determination of reference standards/thresholds according to, for example, the andrological pathology presented by the infertile man, nor standards/thresholds for the selection of a particular ART technique.

2. Use of artificial intelligence in sperm analysis

Evaluation of the infertile male is governed by the data obtained from conventional semen parameters, which have limited ability to assess male fertility despite the intensive laboratory skills needed. This has prompted scientists to develop computational methods to replace manual alternatives while studying the possibility of incorporating artificial intelligence (AI) within the scope of andrology [42-46]. The unprecedented increase in complex medical data surpasses the capacity of the basic statistical models to deduce the desired information. Hence, AI has been considered for using different complex algorithms in appraising a relationship between different variables related to fertility [42]. In a multi-institutional study by Ory et al [47], a machine learning (ML) model successfully predicted subsequent upgrades in sperm parameters in 87% of men (area under the curve [AUC]=0.72) following varicocele repair. Another example of an AI model is Bemaner (Shenzhen Createcare Technology Co.), a smartphone application that measures sperm motility at home, capturing and uploading videos assessed by an AI algorithm of image recognition. Bemaner's results of sperm analysis were compared to grades offered by experienced andrologists and showed a strong correlation between total and motile sperm concentration ($r=0.65$, $p<0.001$; $r=0.84$, $p<0.001$, respectively) and percentage of motility ($r=0.90$, $p<0.001$) [48]. However, there is a concern of providing false reassurance without a complete formal SA including findings such as the presence of leukocytes, sperm agglutination, or other microscopic clues of potential pathology. The same principle has been explored by Kobori et al [49], who used a computer-assisted semen analyzer for both comparison and validation.

Another recent advance is the use of AI methods which are particularly objective and suitable for video images [50,51]. These correspond to ML, a sub-field of AI. These methods promise to improve intracytoplasmic sperm injection (ICSI) by guiding clinicians to objectively select the optimum sperm [51,52]. Some AI devices have been developed to analyze sperm morphology [53-56]. Recent morphological assessment models based on unstained sperm images are being developed to improve the ICSI technique by classifying images in real-time [57,58]. In a recent review, it was stated that the currently existing models are restricted in their capabilities, necessitating the need to develop specific models tailored to andrology [59]. While the implementation of AI in andrology holds promise, it encounters various challenges. Firstly, data sources often lack accuracy or completeness. Secondly, the absence of standardized protocols hinders the deployment of the limited number of available AI models. Moreover, the approval process for AI in medical applications lacks consistency across different governing bodies. Thirdly, there is a concern that AI might restrict patient care autonomy. Currently, most clinicians place importance on a patient-focused and evidence-based approach to decision-making. However, a recent survey involving German healthcare providers indicates an inclination to embrace AI technology in medical care [60]. Fourthly, the cost of developing and validating AI models in andrology poses a significant challenge due to limited funding availability. This is especially pronounced considering the relatively small size of the andrology specialty compared to other medical fields. Lastly, ethical concerns arise regarding the suitability of AI models for all patients, particularly when considerable individual variations are expected. Indeed, an ethical dilemma arises with the introduction of novel and ex-

pensive tools, for example, in concerning the fair allocation of payment and beneficiaries of this technology, and in considering disparities in insurance and financial resources across the medical landscape in various countries [59].

3. Home testing of semen

Many males experience significant pressure when asked to deliver a semen sample in the laboratory premises, a fact that encouraged the idea of developing semen home collection kits, offering a more convenient way of collection. Many products have been implemented and studied to replace the conventional lab-based collection model [43]. The Food and Drug Administration has approved many at-home sperm testing products based on their accuracy and ease of use, including SpermCheck®, YO®, and Trak®. The SpermCheck® utilizes sperm-specific monoclonal antibodies and offers an accuracy of 97% to 98% when compared with trained laboratory professionals [43,61]. The YO® system connects a smartphone camera to the sample examination station and evaluates motile sperm concentration with an accuracy between 97.2% and 98.3%, according to the type of smartphone used [62]. The Trak® system comprises of a portable device used to assess sperm count using centrifugal motion and claims an accuracy of 93.3%, 82.4%, and 95.5% for results categorized as ≤15 million/mL, 15–55 million/mL, and >55 million/mL, respectively [43,63]. On the other hand, the Micra Sperm Test is a thirty-minute home-based sperm testing product measuring semen volume, sperm count and motility, but the results are subjected to variability [43,62].

4. Whole genome testing

Many genetic defects have been identified following the innovative emergence of next-generation sequencing (NGS) [64]. The exome, which represents 1% of the human genome, consists of 180,000 exons [65]. Currently, complex whole exome sequencing represents the diagnostic tool of choice, but it is believed that whole exome sequencing (WES) will be replaced by whole genome sequencing (WGS), which has a lower cost and incorporates practical software facilitating the interpretation of results [66]. Practically, this has helped in the understanding of several male infertility states; for instance, several genes have been identified in the context of non-obstructive azoospermia (NOA), including FANCA, PLK4, WKN3, MEI1, ADAD2, and TEX11 [66,67].

5. Epigenetic markers

Many epigenetic markers have been considered in assessing the presence of active spermatogenesis among NOA patients. For instance, ESX1 transcript was identified in approximately 95% (62 out of 65 samples) of males with the presence of spermatogenesis in testicular tissue [68]. Additionally, a study by Yao et al [69] demonstrated 396, 395, and 378 microRNAs that were differentially expressed in the spermatogonia, pachytene spermatocytes, and round spermatids, respectively, between NOA and patients with obstructive azoospermia. It is suggested that epigenetic markers may help resolve much of the current uncertainty that surrounds the prediction of the presence or absence of spermatogenesis in azoospermic men.

6. Seminal proteomics

Studying the seminal plasma is a novel approach that supports the management of male infertility since it is rich in protein biomarkers at a concentration of 35–55 mg/mL, with semenogelins and kallikrein 3 being two examples of highly abundant seminal proteins [70,71]. This is, however, challenged by the variability in protein concentrations between individuals [71]. From a clinical perspective, Batruch et al [72] identified different seminal protein expression profiles between patients with NOA and their fertile counterparts. An altered seminal plasma proteomic profile is also found in patients with varicocele, correlated with increased ROS generation and up-regulation of antioxidant systems [73,74]. Sixty-four and 31 proteins were expressed in bilateral and unilateral varicocele patients, respectively, which reflected the varicocele severity and its impact on seminal parameters [75]. Seminal protein RNAs could predict the presence of spermatozoa in patients with NOA in certain instances, as demonstrated for miR-192a [76] and hsa-circ-0000116 [77]. Therefore, RNAs that regulate germ cell apoptosis and are involved in spermatogenesis may also play a role in predicting sperm retrieval. The efficacy of ECM1, TEX101, and LGALS3BP in predicting TESE outcomes in patients with NOA has already been examined, providing more support for SP proteomics. Very intriguingly, ECM1 could also be important for ART result prediction [78]. Additional studies on larger populations are

warranted to validate the role of protein biomarkers in the clinical practice of male infertility [79].

7. Radiomics

Radiomics involves the extraction of numerical values from radiological images, thus offering a more comprehensive analysis that is beyond the simple visual capacity [80,81]. A pilot study by De Santi et al [82] compared scrotal ultrasonographic findings and testicular function represented by semen parameters (sperm concentration, total sperm number, total motility, progressive motility, and sperm morphology) and reproductive hormones (luteinizing hormone [LH], follicle-stimulating hormone [FSH], and total testosterone). The results showed that ultrasound-related textural features were correlated and predicted sperm concentrations and total counts, total and progressive motility, morphology, and serum gonadotropins but did not correlate with serum total testosterone levels.

In a recent study on ten males with NOA, it was observed that choline and creatine were the most pronounced metabolite peaks seen following spectroscopic examination of five males with NOA who were positive for sperm during microTESE [83]. Testicular normalized apparent diffusion coefficient (ADC) derived from the conventional mono-exponential model is a parameter that reflects the water diffusion motion, which is primarily associated with the cell density of the tissue and extracellular space [84]. "In the testis, compact interstitial and connective tissue, and seminiferous tubules restrict water diffusion, thus affecting the ADC. This can make ADC a useful diagnostic tool. A study on 20 subjects with NOA found a significanty higher ADC in NOA males who had foci of advanced spermatogenesis with a Johnsen score ≥8 [85]."

RELATIONSHIP BETWEEN A MALE'S FERTILITY STATUS AND GENERAL HEALTH

In recent years, general health status is gaining increasing clinical attention in the male reproductive setting. Looking beyond the scope of reproduction and assessing the general well-being of the patient is, therefore, a crucial aspect of the management of infertile males. A Swedish population-based study compared a total of 101,331 males diagnosed with infertility or infertility-related diagnosis with 2,762,254 fertile males and found that the risk of death below the age of 30 years was higher among males diagnosed with infertility (adjusted hazard ratio [HR]=3.26; 95% confidence interval [CI]=2.42–4.41), which was explained by the occurrence of malignancy that was diagnosed before infertility [86]. Thus, it is suggested that infertility can be regarded as a predictor of mortality and morbidity among males [87,88]. In a systematic review and meta-analysis comparing fertile to infertile males, male infertility has been associated with an increased risk of death [89]. In another study, medical comorbidities, including hypertension and hyperlipidemia, were significantly higher in infertile subjects (21.7%) compared to fertile counterparts (9.1%) [90].

A cross-sectional study on more than 9,000 males has shown that comorbidities, including cardiovascular diseases, were higher among patients with low sperm count, motility, and volume [91,92]. Patients with hypertension can be more prone to having abnormal semen parameters [93]. Another study on 32,442 males observed an association between abnormal sperm parameters and testicular malignancy [94]. The development of testicular malignancy was three times higher among infertile males compared to fertile subjects (HR=2.8; 95% CI=1.3–6.0) [95]. Therefore, clinicians assessing infertile men must also consider their general health [96]. Management of the infertile male should be directed towards not only specific therapy to improve his fertility but also therapy for nonspecific medical comorbidities that may influence his fertility, general health, and life expectancy as well.

PERSONALIZED MEDICINE AND MALE INFERTILITY

Personalized medicine is increasingly used for the treatment of various diseases and disorders. For male infertility, this approach can be delivered in different ways, including stem cell therapy, gene therapy, and nanoparticle drug delivery.

There is growing interest in induced pluripotent stem cells (iPSCs) and mesenchymal stem cells for their potential application in reproductive medicine, particularly in cases of azoospermia-related infertility [97]. The embryonic stem cells (ESC) represent a turning point in regenerative medicine thanks to their unlimited self-renewal properties and, above all, differentiation into ectoderm, endoderm, and mesoderm. An interest-

ing study documented the possibility of developing functional sperm using a sperm-deficient mouse model ($Kit^{w}/Kitw^{v}$) through gene-repaired ESC isolated from cloned blastocysts originating from nuclear transferred somatic cells (ntESC) using gene repair technology [98].

The CRISPR/Cas9 system has enabled reproductive research on gene repair. The CRISPR/Cas9 method allows for modifying the nucleic acids that constitute the genome of all living organisms. The CRISPR/Cas9 system can be used, to generate knockout mouse (KO) mice rapidly and for more complex gene manipulations [99].

The cut-and-sew mechanism of the genome makes it possible to identify a faulty DNA locus and substitute it with a functioning sequence, thereby reverting the infertility condition. By generating KO mouse lines using the CRISPR/Cas9 enzyme, Lu et al [99] were able to analyze the function of 30 testis-enriched genes and four ubiquitously expressed genes involved in male reproduction. The KO males exhibited normal fecundity, suggesting that these 34 genes are expendable on their own for male fertility.

In addition to the genetic approaches being developed, some men will benefit from supplementary medical therapy. The action of antioxidants and their effectiveness in improving the functional capacity of spermatozoa and other parameters of semen is controversial. However, a recent systematic review and meta-analysis of randomized controlled trials demonstrated that antioxidant therapies seem to improve spontaneous pregnancy rate and conventional sperm parameters [100] and measurement of ROS levels may help determine appropriate candidates for antioxidant therapy.

In recent years, advances in nanotechnology have allowed the administration of specific drugs. Solid lipid nanoparticles were first developed in 1990 and are characterized by their submicron size [101]. Together with other characteristics relating to the composition of the matrix, these nanoparticles have the potential to be released in the target area in a precise and prolonged manner [102]. These scientific advances are widely utilized in the veterinary field, but their use for treating infertility in humans has not yet been fully established.

MALE FERTILITY PRESERVATION

Fertility preservation has progressed extensively using innovations in cryobiology for cryopreserving spermatozoa [103]. Different techniques of sperm cryopreservation have been used for ICSI including slow-freezing and vitrification. Slow-freezing is the conventional technique and can result in the formation of ice crystals which may cause damage to the sperm cytoskeleton, membrane, and DNA [104-107]. The technique of vitrification for sperm cryopreservation refers to ultrafast freezing of a small volume of semen with direct contact with contaminant-free liquid nitrogen which reduces osmotic damage by preventing ice formation. Vitrification has been correlated with higher recovery rates and motility [108,109] and lower SDF [110] as compared to slow freeze. However, the 6th edition of the WHO manual for SA recommends that sperm vitrification should be considered an experimental procedure as improved post-thaw semen parameters after vitrification in comparison to conventional cryopreservation techniques have limited data to support it [34].

Despite these innovations, it has been argued that a series of epigenetic modifications may occur secondary to cryopreservation, including changes in mRNA expression [111]. Cryopreservation is used following testicular sperm retrieval in infertile azoospermic males, where a limited number of sperm are retrieved [112], or as fertility preservation before chemo-radiotherapy. Fertility preservation is much more challenging in the context of pre-pubertal boys suffering from cancer who will need different oncologic therapies. Since spermatogenesis occurs at puberty, harvesting and cryopreserving spermatogonial stem cells before undergoing therapy is being studied as a possible fertility-preserving option for pre-pubertal patients, where the preserved tissue could either be used for autologous transplantation or for *in vitro* induction of spermatogenesis [113]. These techniques have been successfully performed in a mouse model, where the cryopreserved harvested tissues were cultured after thawing and resulted in complete spermatogenesis, and sperm were successfully used for ICSI [114,115].

Cryopreservation of testicular tissue in pre-pubertal boys has been discussed for more than 20 years [116,117]. The rationale behind the cryopreservation of testicular tissue is to restore spermatogenesis in adulthood. Overall, patient survival from childhood cancer has dramat-

ically increased with the development of chemo- and radiotherapy regimens. In most European countries, overall five-year childhood cancer survival is estimated at more than 80% and approximately 500,000 childhood cancer survivors will expect to be a father [118]. It is simple to collect spermatozoa in adults before any gonadotoxic treatment and use them in future ARTs [119]; however, for pre-pubertal boys, there is no established method defined to preserve and restore spermatogenesis [120].

Autologously engrafted frozen-thawed ovarian cortex has successfully led to more than 100 live births in female cancer survivors worldwide [121]. Theoretically, the analog method with testicular tissue engraftment in pre-pubertal boys is also expected to restore spermatogenesis and lead to successful pregnancies. Achieving spermatogenesis by orthotopic and ectopic transplantation of pre-pubertal testicular tissue has been demonstrated in mice [122]. In another study, from two juvenile monkey testis xenografts, six healthy monkeys were produced by ICSI [123]. In 2019, a milestone paper showed the possibility of spermatogenesis restoration when cryopreserved and fresh pre-pubertal testis tissues from rhesus monkeys were subjected to autologous grafting beneath the skin of the back or scrotum [124]. In the latter study, sperm derived from scrotal skin was used in ICSI, and a Grady baby ('graft-derived baby') was born. Although this method seems more promising for advancing fertility preservation in pre-pubertal boys to a clinical stage, several concerns, including optimal testicular tissue size to be transplanted, the ideal age of transplantation, and post-grafting time for sperm extraction, should first be eliminated [120].

FUTURE OF STEM CELLS IN MALE INFERTILITY

Strategies to restore fertility for pre-pubertal boys include spermatogonial stem cell transplantation (SSCT), testicular tissue engraftment, and *in vitro* spermatogenesis [120,125]. Autologous SSCT is one of the most studied methods for restoring fertility in pre-pubertal boys. The proof of concept study in mice was published in 1994 [126]; thereafter, in many species, SSCT was shown successfully to restore spermatogenesis [127]. The first study applying SSCT to humans was performed in 1999; however, the results of this study have never been reported [128]. Since then, no SSCT attempts for humans have been reported as several major concerns limit the application of SSCT in a real-life setting.

Culturing human SSCs without any Xeno product is the first issue that needs to be overcome. Using animal components in cell culture introduces the risk of contaminating cells with pathogens, making them inappropriate for medical use. Recently, a xeno-free culture method has been developed to propagate SSCs from infant boys using human platelet lysate and human serum albumin replacing fetal bovine serum and bovine serum albumin [129]. Some other components have also been proposed to replace animal-derived components, such as: bovine serum albumin or fetal bovine serum [130]. However, further studies are required to establish an optimum xeno-free media to expand SSCs and use them in future cultural practices. In addition to optimizing SSC culture with xeno-free components, the risk of potential malignant cell transmission and defining the optimum technique are the other leading limitations of SSCT. Although several sorting strategies have been proposed, evidence is inconsistent and unconvincing regarding eliminating malignant cells from SSCs [131,132]. Similarly, data are inconclusive regarding the optimal SSCT technique, as more studies are needed regarding the optimal transplantation site, optimum cell count, and ideal hydrostatic pressure [133-135].

In vitro maturation of SSCs to spermatozoa has been studied for over a century. In 1999, *in vitro* maturation of testicular samples from males with premeiotic maturation arrest led to successful pregnancy and live birth [136]. However, other groups could not replicate this study due to a lack of definitive protocols. Considering that the SSCs' self-renewal and differentiation need a stem cell niche (testicular architecture and somatic support), establishing the optimum culture system is difficult. The development of biomaterials and nanotechnology may help the progress of *in vitro* spermatogenesis into the clinical stage [137-139].

It is possible to derive human induced pluripotent stem cells, also known as hiPSCs, from the somatic cells of patients. The *in vitro* development of functional germ cells from patient-specific induced pluripotent stem cells (iPSCs) may give new therapeutic strategies for couples who are unable to have children [140]. Idiopathic infertility patients' somatic cells (such as skin

fibroblast, keratinocyte, peripheral blood mononuclear cells, or renal tubular cells in urine) are reprogrammed into iPSCs, and these iPSCs are subsequently differentiated into male germ cells using a variety of techniques [141-145]. The process is called "differentiation." iPSCs may, if necessary, undergo genome editing to fix known genetic flaws. These cells have the potential to be utilized for *in vitro* disease modeling, research on tissue regeneration, and cell-based therapeutic treatment. In disease modeling, comparing cells obtained from patients to those derived from normal individuals can reveal unique insights into the underlying mechanisms that cause idiopathic male infertility. However, the chemical pathways underpinning the formation of male germ cells remain poorly known. The employment of hiPSCs in reproductive medicine and fundamental research would benefit greatly from a greater understanding of human germ cell development [140].

THE ART OF ART

Among the most widely used treatment methods, ARTs have become the gold standard in medically assisted reproductive medicine. Not surprisingly, the keyword intracytoplasmic sperm injection "ICSI" has been cited over 13,000 times in 30 years, according to a Scopus search as of June 2022.

Despite the continuous progress made by ART clinics and laboratories, ICSI live birth rates vary between 12.3% and 46.5% per oocyte retrieval cycle (attempt), with an average cumulative retrieval live birth rate of less than 30% [146]. For this reason, over the past 30 years, several "variants" of ICSI have been described. Researchers have focused on methods of sperm selection before injection. These include intracytoplasmic morphologically selected sperm injection (IMSI) using differential interferential contrast and high magnification, physiologic ICSI (PICSI) with hyaluronic acid/hyaluronan, magnetic-activated cell sorting (MACs) techniques, the zeta potential sperm selection process, zona pellucida-bound sperm selection, and microfluidic sperm sorting cells. Recent meta-analyses are inconclusive about the efficacy of these ICSI variants (compared to conventional ICSI), as the quality of the studies included in these meta-analyses are ranked to be moderate to low [147,148]. It should be noted, however, that the trend is towards improved pregnancy rates with certain variants of ICSI, such as IMSI [148]. Additionally, some of these techniques can be useful in certain indications that can be determined after careful andrological evaluation of the male partner. Indeed, selecting the best spermatozoon, the one with the most intact and least fragmented DNA in a patient with non-treated risk factors for SDF, for example, is pointless. The initial evaluation of infertile males and the management of the causes of infertility will undoubtedly be important aspects of the post-ICSI era.

Laser-assisted (LA) sperm selection technique selects immotile but living sperm cells and results in a healthy birth [149]. It was also effective in pentoxifylline-resistant immotile spermatozoa in males with Kartagener's syndrome [150]. Birefringence-based selection technique is another advancement where a light wave is split into two unequally reflected waves using an optically anisotropic medium. With the use of polarized light microscopy, mature and viable spermatozoa can be selected. The technique has been found superior to the hypo-osmotic swelling test with higher pregnancy rates in TESE/ICSI cycles [151,152]. On the other hand, fluorescence-activated cell sorting (FACS) isolates living sperm cells labeled with fluorophore-conjugated antibodies from seminal fluid when excited by a laser beam [153]. The technology has been implemented recently in sperm isolation after TESE from males with NOA. However, the cost of the procedure, the possible sperm loss and the time constraint have limited this technology [154].

AI FOR ANDROLOGICAL SURGERIES

AI has the potential to play a significant role in andrological surgeries as well. AI can be used in andrological surgery in several ways. One way is through the use of ML models, which can be guided by AI algorithms to predict surgery outcomes. In a study by Ory et al [47], using pre-operative hormonal, clinical, and sperm analysis data, a ML model successfully predicted clinically significant improvement in post-varicocelectomy sperm parameters. Also, there were some attempts for predicting sperm retrieval in mTESE for NOA patients [155,156]. In a paper by Zeadna et al [156], a model including LH, FSH, testosterone, testicular size, semen volume, age, BMI, and ethnicity as candidate predictors were able to predict sperm retrieval rate with moderate accuracy (AUC=0.8). However, this paper was criticized for its low number of candidate predictors, small

sample size, selection bias, and surgical technique used for sperm retrieval [157]. Also, deep neural networks are used to segment the penile shaft before assessment and it provides accuracy on par with manual examination for patients with penile curvature [158]. It seems that the ML models in andrological surgeries have a long way to be used in the andrological surgeries.

THE FUTURE OF ANDROLOGISTS

Undoubtedly, the role of the andrologist will expand in the coming years with international initiatives already underway. For example, under the auspices of the European Society of Human Reproduction and Embryology (ESHRE), a group has been created to improve global research and management of male fertility and infertility [159]. In the same year, a global group of andrologists was created, collectively known as the Global Andrology Forum (GAF) (https://globalandrologyforum.com) [160]. This group currently has about 700 members from 84 countries. The goals of the GAF include collaborations between andrologists from around the world, addressing questions and controversies in andrology, training researchers and physicians in scientific research in andrology, and making andrology a field of research care and training on its own. Several training courses were held online during the COVID pandemic, which attracted hundreds of researchers and clinicians interested in andrology [161,162].

CONCLUSIONS

Male infertility is a common problem and a significant source of stressful life. To determine the optimal treatment for male infertility, a comprehensive, individualized diagnostic workup is warranted to determine the underlying cause. The development of genetic testing and the use of epigenetic markers, seminal proteomes, and radiomics offers hope for understanding the etiopathogenesis of male infertility. In addition, advancements in male fertility preservation tools provide hope to restore the fertility potential of male patients with cancer undergoing gonadotoxic therapies. In addition, the future application of AI in the infertility practice may help establish a definitive diagnosis for many infertility cases and provide prognostic value for sperm extraction and reproductive outcome under natural and assisted conditions. Sincere efforts of the professional organizations of andrology will enhance the spread of andrological knowledge and reduce the gap between the research field and clinical practice.

Conflict of Interest

The authors have nothing to disclose.

Funding

None.

Acknowledgements

We are grateful to Dr. Manaf Al Hashimi, MD (UAE), Dr. Wael Zohdy, MD (Egypt), and Prof. Umut Çağın Arı, PhD, DVM (Turkey) for critically reviewing our manuscript. The help of Dr. Damayanthi Durairajanayagam, PhD (Malaysia) in editing our manuscript is appreciated.

Author Contribution

Conceptualization: AA, MG, FB, GIR, HK. Methodology: AA, MG, FB, GIR, HK. Investigation: MG, FB, GIR, HK. Supervision: AA, R Saleh. Project administration: AA. Writing – original draft preparation: MG, FB, GIR, HK. Writing – review & editing: MG, GIR, HK, FB, R Saleh, EC, PK, TM, R Shah, AA. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. World Health Organization (WHO). WHO laboratory manual for the examination and processing of human semen. 6th ed. WHO; 2021.
2. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update 2015;21:411-26.
3. Sinclair S. Male infertility: nutritional and environmental considerations. Altern Med Rev 2000;5:28-38.
4. Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. Clin Biochem 2018;62:2-10.
5. Alsaikhan B, Alrabeeah K, Delouya G, Zini A. Epidemiology of varicocele. Asian J Androl 2016;18:179-81.
6. Baazeem A. Varicocele: how this condition and its management affects men's health. World J Meta Anal 2014;2:17-23.
7. Agarwal A, Cannarella R, Saleh R, Boitrelle F, Gül M, Toprak

T, et al. Impact of varicocele repair on semen parameters in infertile men: a systematic review and meta-analysis. World J Mens Health 2023;41:289-310.
8. Panach-Navarrete J, Morales-Giraldo A, Ferrandis-Cortés C, García-Morata F, Pastor-Lence JC, Martínez-Jabaloyas JM. Is there a relationship between varicocele and testosterone levels? Aging Male 2020;23:592-8.
9. Chen X, Yang D, Lin G, Bao J, Wang J, Tan W. Efficacy of varicocelectomy in the treatment of hypogonadism in subfertile males with clinical varicocele: a meta-analysis. Andrologia 2017;49:e12778.
10. Fallara G, Cazzaniga W, Boeri L, Capogrosso P, Candela L, Pozzi E, et al. Male factor infertility trends throughout the last 10 years: report from a tertiary-referral academic andrology centre. Andrology 2021;9:610-7.
11. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. Eur Urol 2016;70:635-45.
12. Holmboe SA, Priskorn L, Jensen TK, Skakkebaek NE, Andersson AM, Jørgensen N. Use of e-cigarettes associated with lower sperm counts in a cross-sectional study of young men from the general population. Hum Reprod 2020;35:1693-701.
13. Amiri M, Ramezani Tehrani F. Potential adverse effects of female and male obesity on fertility: a narrative review. Int J Endocrinol Metab 2020;18:e101776.
14. Liu Y, Ding Z. Obesity, a serious etiologic factor for male subfertility in modern society. Reproduction 2017;154:R123-31.
15. Katib A. Mechanisms linking obesity to male infertility. Cent European J Urol 2015;68:79-85.
16. de Castro Barbosa T, Ingerslev LR, Alm PS, Versteyhe S, Massart J, Rasmussen M, et al. High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. Mol Metab 2015;5:184-97.
17. Cannarella R, Caruso M, Condorelli RA, Timpanaro TA, Caruso MA, La Vignera S, et al. Testicular volume in 268 children and adolescents followed-up for childhood obesity-a retrospective cross-sectional study. Eur J Endocrinol 2023;188:331-42.
18. Pizzorno J. Environmental toxins and infertility. Integr Med (Encinitas) 2018;17:8-11.
19. Cannarella R, Gül M, Rambhatla A, Agarwal A. Temporal decline of sperm concentration: role of endocrine disruptors. Endocrine 2023;79:1-16.
20. Cescon M, Chianese R, Tavares RS. Environmental impact on male (in)fertility via epigenetic route. J Clin Med 2020;9:2520.
21. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020;368:m1091. Erratum in: BMJ 2020;368:m1295.
22. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506. Erratum in: Lancet 2020;395:496.
23. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ 2020;368:m606. Erratum in: BMJ 2020;368:m792.
24. Peckham H, de Gruijter NM, Raine C, Radziszewska A, Ciurtin C, Wedderburn LR, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. Nat Commun 2020;11:6317.
25. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol 2022;23:3-20.
26. Collins AB, Zhao L, Zhu Z, Givens NT, Bai Q, Wakefield MR, et al. Impact of COVID-19 on male fertility. Urology 2022;164:33-9.
27. Tur-Kaspa I, Tur-Kaspa T, Hildebrand G, Cohen D. COVID-19 may affect male fertility but is not sexually transmitted: a systematic review. F S Rev 2021;2:140-9.
28. Pan PP, Zhan QT, Le F, Zheng YM, Jin F. Angiotensin-converting enzymes play a dominant role in fertility. Int J Mol Sci 2013;14:21071-86.
29. Donders GGG, Bosmans E, Reumers J, Donders F, Jonckheere J, Salembier G, et al. Sperm quality and absence of SARS-CoV-2 RNA in semen after COVID-19 infection: a prospective, observational study and validation of the SpermCOVID test. Fertil Steril 2022;117:287-96.
30. Tremellen K. Oxidative stress and male infertility--a clinical perspective. Hum Reprod Update 2008;14:243-58.
31. 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.
32. Kavoussi PK, Gilkey MS, Machen GL, Kavoussi SK, Dorsey C. Varicocele repair improves static oxidation reduction potential as a measure of seminal oxidative stress levels in infertile men: a prospective clinical trial using the MiOXSYS system. Urology 2022;165:193-7.
33. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. Fertil Steril 2021;115:54-61.
34. Boitrelle F, Shah R, Saleh R, Henkel R, Kandil H, Chung E, et

al. The sixth edition of the WHO manual for human semen analysis: a critical review and SWOT analysis. Life (Basel) 2021;11:1368.
35. Agarwal A, Majzoub A, Baskaran S, Panner Selvam MK, Cho CL, Henkel R, et al. Sperm DNA fragmentation: a new guideline for clinicians. World J Mens Health 2020;38:412-71.
36. Agarwal A, Farkouh A, Parekh N, Zini A, Arafa M, Kandil H, et al. Sperm DNA fragmentation: a critical assessment of clinical practice guidelines. World J Mens Health 2022;40:30-7.
37. Esteves SC, Zini A, Coward RM, Evenson DP, Gosálvez J, Lewis SEM, et al. Sperm DNA fragmentation testing: summary evidence and clinical practice recommendations. Andrologia 2021;53:e13874.
38. Farkouh A, Finelli R, Agarwal A. Beyond conventional sperm parameters: the role of sperm DNA fragmentation in male infertility. Minerva Endocrinol (Torino) 2022;47:23-37.
39. Tharakan T, Bettocchi C, Carvalho J, Corona G, Jones TH, Kadioglu A, et al.; EAU Working Panel on Male Sexual Reproductive Health. European Association of Urology guidelines panel on male sexual and reproductive health: a clinical consultation guide on the indications for performing sperm DNA fragmentation testing in men with infertility and testicular sperm extraction in nonazoospermic men. Eur Urol Focus 2022;8:339-50.
40. Krenz H, Gromoll J, Darde T, Chalmel F, Dugas M, Tüttelmann F. The male fertility gene atlas: a web tool for collecting and integrating OMICS data in the context of male infertility. Hum Reprod 2020;35:1983-90.
41. Omolaoye TS, Omolaoye VA, Kandasamy RK, Hachim MY, Du Plessis SS. Omics and male infertility: highlighting the application of transcriptomic data. Life (Basel) 2022;12:280.
42. Wang R, Pan W, Jin L, Li Y, Geng Y, Gao C, et al. Artificial intelligence in reproductive medicine. Reproduction 2019;158:R139-54.
43. Yu S, Rubin M, Geevarughese S, Pino JS, Rodriguez HF, Asghar W. Emerging technologies for home-based semen analysis. Andrology 2018;6:10-9.
44. Holt W, Watson P, Curry M, Holt C. Reproducibility of computer-aided semen analysis: comparison of five different systems used in a practical workshop. Fertil Steril 1994;62:1277-82.
45. Lamb DJ, Niederberger CS. Artificial intelligence in medicine and male infertility. World J Urol 1993;11:129-36.
46. Mahmoud AM, Gordts S, Vereecken A, Serneels A, Campo R, Rombauts L, et al. Performance of the sperm quality analyser in predicting the outcome of assisted reproduction. Int J Androl. 1998 Feb;21(1):41-6.
47. Ory J, Tradewell MB, Blankstein U, Lima TF, Nackeeran S, Gonzalez DC, et al. Artificial intelligence based machine learning models predict sperm parameter upgrading after varicocele repair: a multi-institutional analysis. World J Mens Health 2022;40:618-26.
48. Tsai VF, Zhuang B, Pong YH, Hsieh JT, Chang HC. Web- and artificial intelligence-based image recognition for sperm motility analysis: verification study. JMIR Med Inform 2020;8:e20031.
49. Kobori Y, Pfanner P, Prins GS, Niederberger C. Novel device for male infertility screening with single-ball lens microscope and smartphone. Fertil Steril 2016;106:574-8.
50. Hicks SA, Andersen JM, Witczak O, Thambawita V, Halvorsen P, Hammer HL, et al. Machine learning-based analysis of sperm videos and participant data for male fertility prediction. Sci Rep 2019;9:16770.
51. Riegler MA, Stensen MH, Witczak O, Andersen JM, Hicks SA, Hammer HL, et al. Artificial intelligence in the fertility clinic: status, pitfalls and possibilities. Hum Reprod 2021;36:2429-42.
52. You JB, McCallum C, Wang Y, Riordon J, Nosrati R, Sinton D. Machine learning for sperm selection. Nat Rev Urol 2021;18:387-403.
53. Chandra S, Gourisaria MK, Gm H, Konar D, Gao X, Wang T, et al. Prolificacy assessment of spermatozoan via state-of-the-art deep learning frameworks. IEEE Access 2022;10:13715-27.
54. Ilhan HO, Serbes G. Sperm morphology analysis by using the fusion of two-stage fine-tuned deep networks. Biomed Signal Process Control 2022;71:103246.
55. Ilhan HO, Sigirci IO, Serbes G, Aydin N. A fully automated hybrid human sperm detection and classification system based on mobile-net and the performance comparison with conventional methods. Med Biol Eng Comput 2020;58:1047-68.
56. Movahed RA, Mohammadi E, Orooji M. Automatic segmentation of sperm's parts in microscopic images of human semen smears using concatenated learning approaches. Comput Biol Med 2019;109:242-53.
57. Abbasi A, Miahi E, Mirroshandel SA. Effect of deep transfer and multi-task learning on sperm abnormality detection. Comput Biol Med 2021;128:104121.
58. Javadi S, Mirroshandel SA. A novel deep learning method for automatic assessment of human sperm images. Comput Biol Med 2019;109:182-94.
59. Ghayda RA, Cannarella R, Calogero AE, Shah R, Rambhatla A, Zohdy W, et al.; Global Andrology Forum. Artificial intelligence in andrology: from semen analysis to image diagnostics. World J Mens Health 2023. doi: 10.5534/wjmh.230050

[Epub]
60. Maassen O, Fritsch S, Palm J, Deffge S, Kunze J, Marx G, et al. Future medical artificial intelligence application requirements and expectations of physicians in German university hospitals: web-based survey. J Med Internet Res 2021;23:e26646.
61. Coppola MA, Klotz KL, Kim KA, Cho HY, Kang J, Shetty J, et al. SpermCheck fertility, an immunodiagnostic home test that detects normozoospermia and severe oligozoospermia. Hum Reprod 2010;25:853-61.
62. Onofre J, Geenen L, Cox A, Van Der Auwera I, Willendrup F, Andersen E, et al. Simplified sperm testing devices: a possible tool to overcome lack of accessibility and inconsistency in male factor infertility diagnosis. An opportunity for low- and middle- income countries. Facts Views Vis Obgyn 2021;13:79-93.
63. Schaff UY, Fredriksen LL, Epperson JG, Quebral TR, Naab S, Sarno MJ, et al. Novel centrifugal technology for measuring sperm concentration in the home. Fertil Steril 2017;107:358-64.e4.
64. Sun Y, Ruivenkamp CA, Hoffer MJ, Vrijenhoek T, Kriek M, van Asperen CJ, et al. Next-generation diagnostics: gene panel, exome, or whole genome? Hum Mutat 2015;36:648-55.
65. Kolmykov S, Vasiliev G, Osadchuk L, Kleschev M, Osadchuk A. Whole-exome sequencing analysis of human semen quality in Russian multiethnic population. Front Genet 2021;12:662846.
66. Ghieh F, Barbotin AL, Leroy C, Marcelli F, Swierkowsky-Blanchard N, Serazin V, et al. Will whole-genome sequencing become the first-line genetic analysis for male infertility in the near future? Basic Clin Androl 2021;31:21.
67. Cioppi F, Rosta V, Krausz C. Genetics of azoospermia. Int J Mol Sci 2021;22:3264.
68. Bonaparte E, Moretti M, Colpi GM, Nerva F, Contalbi G, Vaccalluzzo L, et al. ESX1 gene expression as a robust marker of residual spermatogenesis in azoospermic men. Hum Reprod 2010;25:1398-403.
69. Yao C, Yuan Q, Niu M, Fu H, Zhou F, Zhang W, et al. Distinct expression profiles and novel targets of MicroRNAs in human spermatogonia, pachytene spermatocytes, and round spermatids between OA patients and NOA patients. Mol Ther Nucleic Acids 2017;9:182-94.
70. Pilch B, Mann M. Large-scale and high-confidence proteomic analysis of human seminal plasma. Genome Biol 2006;7:R40.
71. Gilany K, Minai-Tehrani A, Savadi-Shiraz E, Rezadoost H, Lakpour N. Exploring the human seminal plasma proteome: an unexplored gold mine of biomarker for male infertility and male reproduction disorder. J Reprod Infertil 2015;16:61-71.
72. Batruch I, Smith CR, Mullen BJ, Grober E, Lo KC, Diamandis EP, et al. Analysis of seminal plasma from patients with non-obstructive azoospermia and identification of candidate biomarkers of male infertility. J Proteome Res 2012;11:1503-11.
73. Panner Selvam MK, Agarwal A, Baskaran S. Proteomic analysis of seminal plasma from bilateral varicocele patients indicates an oxidative state and increased inflammatory response. Asian J Androl 2019;21:544-50.
74. Panner Selvam MK, Samanta L, Agarwal A. Functional analysis of differentially expressed acetylated spermatozoal proteins in infertile men with unilateral and bilateral varicocele. Int J Mol Sci 2020;21:3155.
75. Agarwal A, Sharma R, Durairajanayagam D, Cui Z, Ayaz A, Gupta S, et al. Differential proteomic profiling of spermatozoal proteins of infertile men with unilateral or bilateral varicocele. Urology 2015;85:580-8.
76. Zhi EL, Liang GQ, Li P, Chen HX, Tian RH, Xu P, et al. Seminal plasma miR-192a: a biomarker predicting successful resolution of nonobstructive azoospermia following varicocele repair. Asian J Androl 2018;20:396-9.
77. Lv MQ, Zhou L, Ge P, Li YX, Zhang J, Zhou DX. Over-expression of hsa_circ_0000116 in patients with non-obstructive azoospermia and its predictive value in testicular sperm retrieval. Andrology 2020;8:1834-43.
78. Cannarella R, Barbagallo F, Crafa A, La Vignera S, Condorelli RA, Calogero AE. Seminal plasma transcriptome and proteome: towards a molecular approach in the diagnosis of idiopathic male infertility. Int J Mol Sci 2020;21:7308.
79. Bieniek JM, Drabovich AP, Lo KC. Seminal biomarkers for the evaluation of male infertility. Asian J Androl 2016;18:426-33.
80. Lambin P, Rios-Velazquez E, Leijenaar R, Carvalho S, van Stiphout RG, Granton P, et al. Radiomics: extracting more information from medical images using advanced feature analysis. Eur J Cancer 2012;48:441-6.
81. Gillies RJ, Kinahan PE, Hricak H. Radiomics: images are more than pictures, they are data. Radiology 2016;278:563-77.
82. De Santi B, Spaggiari G, Granata AR, Romeo M, Molinari F, Simoni M, et al. From subjective to objective: a pilot study on testicular radiomics analysis as a measure of gonadal function. Andrology 2022;10:505-17.
83. Karakus C, Ozyurt R. Correlation between high choline metabolite signal in spectroscopy and sperm retrieval chance at micro-TESE. Eur Rev Med Pharmacol Sci 2022;26:1125-30.
84. Hatakenaka M, Soeda H, Yabuuchi H, Matsuo Y, Kamitani T, Oda Y, et al. Apparent diffusion coefficients of breast tumors: clinical application. Magn Reson Med Sci 2008;7:23-9.
85. Tsili AC, Ntorkou A, Goussia A, Astrakas L, Panopoulou E, Sofikitis N, et al. Diffusion tensor imaging parameters in tes-

tes with nonobstructive azoospermia. J Magn Reson Imaging 2018;48:1318-25.
86. Lundberg FE, Johansson AL, Ludvigsson JF. Mortality in 43,598 men with infertility - a Swedish nationwide population-based cohort study. Clin Epidemiol 2019;11:645-57.
87. Del Giudice F, Kasman AM, Ferro M, Sciarra A, De Berardinis E, Belladelli F, et al. Clinical correlation among male infertility and overall male health: a systematic review of the literature. Investig Clin Urol 2020;61:355-71.
88. Glazer CH, Bonde JP, Eisenberg ML, Giwercman A, Hærvig KK, Rimborg S, et al. Male infertility and risk of nonmalignant chronic diseases: a systematic review of the epidemiological evidence. Semin Reprod Med 2017;35:282-90.
89. Del Giudice F, Kasman AM, Chen T, De Berardinis E, Busetto GM, Sciarra A, et al. The association between mortality and male infertility: systematic review and meta-analysis. Urology 2021;154:148-57.
90. Shiraishi K, Matsuyama H. Effects of medical comorbidity on male infertility and comorbidity treatment on spermatogenesis. Fertil Steril 2018;110:1006-11.e2.
91. Eisenberg ML, Li S, Behr B, Pera RR, Cullen MR. Relationship between semen production and medical comorbidity. Fertil Steril 2015;103:66-71.
92. Eisenberg ML, Li S, Cullen MR, Baker LC. Increased risk of incident chronic medical conditions in infertile men: analysis of United States claims data. Fertil Steril 2016;105:629-36.
93. Guo D, Li S, Behr B, Eisenberg ML. Hypertension and male fertility. World J Mens Health 2017;35:59-64.
94. Jacobsen R, Bostofte E, Engholm G, Hansen J, Olsen JH, Skakkebaek NE, et al. Risk of testicular cancer in men with abnormal semen characteristics: cohort study. BMJ 2000;321:789-92.
95. Walsh TJ, Croughan MS, Schembri M, Chan JM, Turek PJ. Increased risk of testicular germ cell cancer among infertile men. Arch Intern Med 2009;169:351-6.
96. Ventimiglia E, Montorsi F, Salonia A. Comorbidities and male infertility: a worrisome picture. Curr Opin Urol 2016;26:146-51.
97. Saha S, Roy P, Corbitt C, Kakar SS. Application of stem cell therapy for infertility. Cells 2021;10:1613.
98. Yuan Y, Zhou Q, Wan H, Shen B, Wang X, Wang M, et al. Generation of fertile offspring from Kit(w)/Kit(wv) mice through differentiation of gene corrected nuclear transfer embryonic stem cells. Cell Res 2015;25:851-63.
99. Lu Y, Oura S, Matsumura T, Oji A, Sakurai N, Fujihara Y, et al. CRISPR/Cas9-mediated genome editing reveals 30 testis-enriched genes dispensable for male fertility in mice. Biol Reprod 2019;101:501-11.
100. Agarwal A, Cannarella R, Saleh R, Harraz AM, Kandil H, Salvio G, et al. Impact of antioxidant therapy on natural pregnancy outcomes and semen parameters in infertile men: a systematic review and meta-analysis of randomized controlled trials. World J Mens Health 2023;41:14-48.
101. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application. Adv Pharm Bull 2015;5:305-13.
102. Liu SZ, Feng DC, Liu ZH, Liang JY, Ren ZJ, Zhou C, et al. Development of nanotechnology in andrology. Transl Androl Urol 2020;9:702-8.
103. Hezavehei M, Sharafi M, Kouchesfahani HM, Henkel R, Agarwal A, Esmaeili V, et al. Sperm cryopreservation: a review on current molecular cryobiology and advanced approaches. Reprod Biomed Online 2018;37:327-39.
104. Isachenko E, Isachenko V, Katkov II, Rahimi G, Schöndorf T, Mallmann P, et al. DNA integrity and motility of human spermatozoa after standard slow freezing versus cryoprotectant-free vitrification. Hum Reprod 2004;19:932-9.
105. O'Connell M, McClure N, Lewis SE. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. Hum Reprod 2002;17:704-9.
106. Ozkavukcu S, Erdemli E, Isik A, Oztuna D, Karahuseyinoglu S. Effects of cryopreservation on sperm parameters and ultrastructural morphology of human spermatozoa. J Assist Reprod Genet 2008;25:403-11.
107. Morris GJ. Rapidly cooled human sperm: no evidence of intracellular ice formation. Hum Reprod 2006;21:2075-83.
108. Herbemont C, Mnallah S, Grynberg M, Sifer C. [Prospective comparison of different techniques for cryopreservation of small numbers of human spermatozoa]. Gynecol Obstet Fertil Senol 2019;47:797-801. French.
109. Karthikeyan M, Arakkal D, Mangalaraj AM, Kamath MS. Comparison of conventional slow freeze versus permeable cryoprotectant-free vitrification of abnormal semen sample: a randomized controlled trial. J Hum Reprod Sci 2019;12:150-5.
110. Slabbert M, du Plessis SS, Huyser C. Large volume cryoprotectant-free vitrification: an alternative to conventional cryopreservation for human spermatozoa. Andrologia 2015;47:594-9.
111. Zeng C, Peng W, Ding L, He L, Zhang Y, Fang D, et al. A preliminary study on epigenetic changes during boar spermatozoa cryopreservation. Cryobiology 2014;69:119-27.
112. Di Santo M, Tarozzi N, Nadalini M, Borini A. Human sperm cryopreservation: update on techniques, effect on DNA integrity, and implications for ART. Adv Urol 2012;2012:854837.
113. Hussein AA, Tran ND, Smith JF. Fertility preservation for boys and adolescents facing sterilizing medical therapy. Transl

Androl Urol 2014;3:382-90.
114. Sato T, Katagiri K, Kubota Y, Ogawa T. In vitro sperm production from mouse spermatogonial stem cell lines using an organ culture method. Nat Protoc 2013;8:2098-104.
115. Yokonishi T, Sato T, Komeya M, Katagiri K, Kubota Y, Nakabayashi K, et al. Offspring production with sperm grown in vitro from cryopreserved testis tissues. Nat Commun 2014;5:4320.
116. Bahadur G, Chatterjee R, Ralph D. Testicular tissue cryopreservation in boys. Ethical and legal issues: case report. Hum Reprod 2000;15:1416-20.
117. Valli-Pulaski H, Peters KA, Gassei K, Steimer SR, Sukhwani M, Hermann BP, et al. Testicular tissue cryopreservation: 8 years of experience from a coordinated network of academic centers. Hum Reprod 2019;34:966-77.
118. Winther JF, Kenborg L, Byrne J, Hjorth L, Kaatsch P, Kremer LC, et al. Childhood cancer survivor cohorts in Europe. Acta Oncol 2015;54:655-68.
119. Trottmann M, Becker AJ, Stadler T, Straub J, Soljanik I, Schlenker B, et al. Semen quality in men with malignant diseases before and after therapy and the role of cryopreservation. Eur Urol 2007;52:355-67.
120. Jensen CFS, Dong L, Gul M, Fode M, Hildorf S, Thorup J, et al. Fertility preservation in boys facing gonadotoxic cancer therapy. Nat Rev Urol 2022;19:71-83.
121. Jadoul P, Guilmain A, Squifflet J, Luyckx M, Votino R, Wyns C, et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Hum Reprod 2017;32:1046-54.
122. Schlatt S, Honaramooz A, Boiani M, Schöler HR, Dobrinski I. Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. Biol Reprod 2003;68:2331-5.
123. Liu Z, Nie YH, Zhang CC, Cai YJ, Wang Y, Lu HP, et al. Generation of macaques with sperm derived from juvenile monkey testicular xenografts. Cell Res 2016;26:139-42.
124. Fayomi AP, Peters K, Sukhwani M, Valli-Pulaski H, Shetty G, Meistrich ML, et al. Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. Science 2019;363:1314-9. Erratum in: Science 2019;364:eaax4999.
125. Dong L, Kristensen SG, Hildorf S, Gul M, Clasen-Linde E, Fedder J, et al. Propagation of spermatogonial stem cell-like cells from infant boys. Front Physiol 2019;10:1155.
126. Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A 1994;91:11298-302.
127. Gul M, Hildorf S, Dong L, Thorup J, Hoffmann ER, Jensen CFS, et al. Review of injection techniques for spermatogonial stem cell transplantation. Hum Reprod Update 2020;26:368-91.
128. Radford J, Shalet S, Lieberman B. Fertility after treatment for cancer. Questions remain over ways of preserving ovarian and testicular tissue. BMJ 1999;319:935-6.
129. Dong L, Gul M, Hildorf S, Pors SE, Kristensen SG, Hoffmann ER, et al. Xeno-free propagation of spermatogonial stem cells from infant boys. Int J Mol Sci 2019;20:5390.
130. Robinson M, Witherspoon L, Willerth S, Flannigan R. A xeno-free media for the in vitro expansion of human spermatogonial stem cells. bioRxiv 2021. doi: 10.1101/2021.06.04.447118 [Epub]
131. Sadri-Ardekani H, Homburg CH, van Capel TM, van den Berg H, van der Veen F, van der Schoot CE, et al. Eliminating acute lymphoblastic leukemia cells from human testicular cell cultures: a pilot study. Fertil Steril 2014;101:1072-8.e1.
132. Dovey SL, Valli H, Hermann BP, Sukhwani M, Donohue J, Castro CA, et al. Eliminating malignant contamination from therapeutic human spermatogonial stem cells. J Clin Invest 2013;123:1833-43.
133. Faes K, Goossens E. Short-term storage of human testicular tissue: effect of storage temperature and tissue size. Reprod Biomed Online 2017;35:180-8.
134. Faes K, Lahoutte T, Hoorens A, Tournaye H, Goossens E. In search of an improved injection technique for the clinical application of spermatogonial stem cell transplantation. Reprod Biomed Online 2017;34:291-7.
135. Faes K, Tournaye H, Goethals L, Lahoutte T, Hoorens A, Goossens E. Testicular cell transplantation into the human testes. Fertil Steril 2013;100:981-8.
136. Tesarik J, Bahceci M, Ozcan C, Greco E, Mendoza C. Restoration of fertility by in-vitro spermatogenesis. Lancet 1999;353:555-6.
137. Alves-Lopes JP, Söder O, Stukenborg JB. Testicular organoid generation by a novel in vitro three-layer gradient system. Biomaterials 2017;130:76-89.
138. Alves-Lopes JP, Söder O, Stukenborg JB. Use of a three-layer gradient system of cells for rat testicular organoid generation. Nat Protoc 2018;13:248-59.
139. Alves-Lopes JP, Stukenborg JB. Testicular organoids: a new model to study the testicular microenvironment in vitro? Hum Reprod Update 2018;24:176-91.
140. Fang F, Li Z, Zhao Q, Li H, Xiong C. Human induced pluripotent stem cells and male infertility: an overview of current progress and perspectives. Hum Reprod 2018;33:188-95.
141. Durruthy Durruthy J, Ramathal C, Sukhwani M, Fang F, Cui J, Orwig KE, et al. Fate of induced pluripotent stem cells following transplantation to murine seminiferous tubules. Hum Mol Genet 2014;23:3071-84.

142. Park TS, Galic Z, Conway AE, Lindgren A, van Handel BJ, Magnusson M, et al. Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. Stem Cells 2009;27:783-95.
143. Eguizabal C, Montserrat N, Vassena R, Barragan M, Garreta E, Garcia-Quevedo L, et al. Complete meiosis from human induced pluripotent stem cells. Stem Cells 2011;29:1186-95.
144. Easley CA 4th, Phillips BT, McGuire MM, Barringer JM, Valli H, Hermann BP, et al. Direct differentiation of human pluripotent stem cells into haploid spermatogenic cells. Cell Rep 2012;2:440-6.
145. Ramathal C, Durruthy-Durruthy J, Sukhwani M, Arakaki JE, Turek PJ, Orwig KE, et al. Fate of iPSCs derived from azoospermic and fertile men following xenotransplantation to murine seminiferous tubules. Cell Rep 2014;7:1284-97.
146. De Geyter C, Calhaz-Jorge C, Kupka MS, Wyns C, Mocanu E, Motrenko T, et al.; European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). ART in Europe, 2014: results generated from European registries by ESHRE: the European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). Hum Reprod 2018;33:1586-601.
147. Lepine S, McDowell S, Searle LM, Kroon B, Glujovsky D, Yazdani A. Advanced sperm selection techniques for assisted reproduction. Cochrane Database Syst Rev 2019;7:CD010461.
148. Teixeira DM, Hadyme Miyague A, Barbosa MA, Navarro PA, Raine-Fenning N, Nastri CO, et al. Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. Cochrane Database Syst Rev 2020;2:CD010167.
149. Chen H, Feng G, Zhang B, Zhou H, Shu J, Gan X. A successful pregnancy using completely immotile but viable frozen-thawed spermatozoa selected by laser. Clin Exp Reprod Med 2017;44:52-5.
150. Ozkavukcu S, Celik-Ozenci C, Konuk E, Atabekoglu C. Live birth after Laser Assisted Viability Assessment (LAVA) to detect pentoxifylline resistant ejaculated immotile spermatozoa during ICSI in a couple with male Kartagener's syndrome. Reprod Biol Endocrinol 2018;16:10.
151. Gianaroli L, Magli MC, Collodel G, Moretti E, Ferraretti AP, Baccetti B. Sperm head's birefringence: a new criterion for sperm selection. Fertil Steril 2008;90:104-12.
152. Ghosh S, Chattopadhyay R, Bose G, Ganesh A, Das S, Chakravarty BN. Selection of birefringent spermatozoa under Polscope: effect on intracytoplasmic sperm injection outcome. Andrologia 2012;44 Suppl 1:734-8.
153. Haas GG Jr, D'Cruz OJ, DeBault LE. Assessment by fluorescence-activated cell sorting of whether sperm-associated immunoglobulin (Ig)G and IgA occur on the same sperm population. Fertil Steril 1990;54:127-32.
154. Mangum CL, Patel DP, Jafek AR, Samuel R, Jenkins TG, Aston KI, et al. Towards a better testicular sperm extraction: novel sperm sorting technologies for non-motile sperm extracted by microdissection TESE. Transl Androl Urol 2020;9(Suppl 2):S206-14.
155. Takeshima T. Development of a machine learning application for intraoperative object detection of positive seminiferous tubules in microdissection testicular sperm extraction for nonobstructive azoospermia. Fertil Steril 2022;118(4 Suppl):E90.
156. Zeadna A, Khateeb N, Rokach L, Lior Y, Har-Vardi I, Harlev A, et al. Prediction of sperm extraction in non-obstructive azoospermia patients: a machine-learning perspective. Hum Reprod 2020;35:1505-14.
157. Caroppo E, Colpi GM. Prediction of sperm retrieval with the aid of machine-learning models cannot help in the management of patients with non-obstructive azoospermia when a less-effective surgical treatment is used. Hum Reprod 2020;35:2872-3.
158. Abbas TO, AbdelMoniem M, Chowdhury MEH. Automated quantification of penile curvature using artificial intelligence. Front Artif Intell 2022;5:954497.
159. Barratt CLR, De Jonge CJ, Anderson RA, Eisenberg ML, Garrido N, Rautakallio Hokkanen S, et al. A global approach to addressing the policy, research and social challenges of male reproductive health. Hum Reprod Open 2021;2021:hoab009.
160. Agarwal A, Saleh R, Boitrelle F, Cannarella R, Hamoda TAA, Durairajanayagam D, et al. The Global Andrology Forum (GAF): a world-wide, innovative, online initiative to bridge the gaps in research and clinical practice of male infertility and sexual health. World J Mens Health 2022;40:537-42.
161. Agarwal A, Leisegang K, Panner Selvam MK, Durairajanayagam D, Barbarosie C, Finelli R, et al. An online educational model in andrology for student training in the art of scientific writing in the COVID-19 pandemic. Andrologia 2021;53:e13961.
162. Agarwal A, Finelli R, Durairajanayagam D, Leisegang K, Sharma R, Gupta S, et al. A web-based global educational model for training in semen analysis during the Covid-19 pandemic. World J Mens Health 2021;39:804-17.