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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-

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 param-

eters 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 significantly 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.

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.

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