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The Renaissance of Male Infertility Management in the Golden Age of Andrology

Aldo E. Calogero[1], Rossella Cannarella[1,2], Ashok Agarwal[3,4], Taha Abo-Almagd Abdel-Meguid Hamoda[5,6], Amarnath Rambhatla[7], Ramadan Saleh[8,9], Florence Boitrelle[10,11], Imad Ziouziou[12], Tuncay Toprak[13], Murat Gul[14], Tomer Avidor-Reiss[15,16], Parviz Kavoussi[17], Eric Chung[18], Ponco Birowo[19], Ramy Abou Ghayda[20], Edmund Ko[21], Giovanni Colpi[22], Fotios Dimitriadis[23], Giorgio Ivan Russo[24], Marlon Martinez[25], Gokhan Calik[26], Hussein Kandil[27], Gianmaria Salvio[28], Taymour Mostafa[29], Haocheng Lin[30], Hyun Jun Park[31,32], Nazim Gherabi[33], Nguyen Ho Vinh Phuoc[34], Nguyen Quang[35,36], Ricky Adriansjah[37], Sandro La Vignera[1], Sava Micic[38], Damayanthi Durairajanayagam[39], Ege Can Serefoglu[40], Vilvapathy Senguttuvan Karthikeyan[41], Priyank Kothari[42], Widi Atmoko[43], Rupin Shah[44]

[1]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy, [2]Glickman Urological & Kidney Institute, Cleveland Clinic, Cleveland, OH, USA, [3]Global Andrology Forum, Moreland Hills, OH, USA, [4]Cleveland Clinic Foundation, Cleveland, OH, USA, [5]Department of Urology, King Abdulaziz University, Jeddah, Saudi Arabia, [6]Department of Urology, Faculty of Medicine, Minia University, Minia, Egypt, [7]Department of Urology, Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI, USA, [8]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag University, Sohag, Egypt, [9]Ajyal IVF Center, Ajyal Hospital, Sohag, Egypt, [10]Reproductive Biology, Fertility Preservation, Andrology, CECOS, Poissy Hospital, Poissy, France, [11]Department of Biology, Reproduction, Epigenetics, Environment and Development, Paris Saclay University, UVSQ, INRAE, BREED, Jouy-en-Josas, France, [12]Department of Urology, College of Medicine and Pharmacy, Ibn Zohr University, Agadir, Morocco, [13]Department of Urology, Fatih Sultan Mehmet Training and Research Hospital, University of Health Sciences, Istanbul, Turkey, [14]Department of Urology, Selcuk University School of Medicine, Konya, Turkey, [15]Department of Biological Sciences, University of Toledo, Toledo, OH, USA, [16]Department of Urology, College of Medicine and Life Sciences, University of Toledo, Toledo, OH, USA, [17]Austin Fertility & Reproductive Medicine/Westlake IVF, Austin, TX, USA, [18]Department of Urology, Princess Alexandra Hospital, University of Queensland, Brisbane, Australia, [19]Department of Urology, Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [20]Urology Institute, University Hospitals, Case Western Reserve University, Cleveland, OH, USA, [21]Department of Urology, Loma Linda University Health, Loma Linda, CA, USA, [22]Next Fertility Procrea, Lugano, Switzerland, [23]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [24]Urology Section, University of Catania, Catania, Italy, [25]Section of Urology, Department of Surgery, University of Santo Tomas Hospital, Manila, Philippines, [26]Department of Urology, Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey, [27]Fakih IVF Fertility Center, Abu Dhabi, UAE, [28]Department of Endocrinology, Polytechnic University of Marche, Ancona, Italy, [29]Department of Andrology, Sexology and STIs, Faculty of Medicine, Cairo University, Cairo, Egypt, [30]Department of Urology, Peking University Third Hospital, Peking University, Beijing, China, [31]Department of Urology, Pusan National University School of Medicine, Busan, Korea, [32]Medical Research Institute of Pusan National University Hospital, Busan, Korea, [33]Faculty of Medicine, Algiers University, Algiers, Algeria, [34]Department of Andrology, Binh Dan Hospital, Ho Chi Minh City, Vietnam, [35]Center for Andrology and Sexual Medicine, Viet Duc University Hospital, Hanoi, Vietnam, [36]Department of Urology, Andrology and Sexual Medicine, University of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam, [37]Department of Urology, Faculty of Medicine Universitas Padjadjaran, Hasan Sadikin General Hospital, Banding, Indonesia, [38]Department of Andrology, Uromedica Polyclinic, Belgrade, Serbia, [39]Department of Physiology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Selangor, Malaysia, [40]Department of Urology, Biruni University School of Medicine, Istanbul, Turkey, [41]Andrology Unit, Department of Urology, Apollo Hospitals, Greams Road, Chennai, India, [42]Department of Urology, B.Y.L Nair Ch Hospital, Mumbai, India, [43]Department Department of Urology, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [44]Division of Andrology, Department of Urology, Lilavati Hospital and Research Centre, Mumbai, India

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Correspondence to: Ashok Agarwal https://orcid.org/0000-0003-0585-1026
Global Andrology Forum, American Center for Reproductive Medicine, 130 West Juniper Lane, Moreland Hills, OH 44022, USA.
Tel: +1-216-312-5829, **E-mail:** agarwaa32099@outlook.com, **Website:** https://www.globalandrologyforum.com

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Infertility affects nearly 186 million people worldwide and the male partner is the cause in about half of the cases. Meta-regression data indicate an unexplained decline in sperm concentration and total sperm count over the last four decades, with an increasing prevalence of male infertility. This suggests an urgent need to implement further basic and clinical research in Andrology. Andrology developed as a branch of urology, gynecology, endocrinology, and, dermatology. The first scientific journal devoted to andrological sciences was founded in 1969. Since then, despite great advancements, andrology has encountered several obstacles in its growth. In fact, for cultural reasons, the male partner has often been neglected in the diagnostic and therapeutic workup of the infertile couple. Furthermore, the development of assisted reproductive techniques (ART) has driven a strong impression that this biotechnology can overcome all forms of infertility, with a common belief that having a spermatozoon from a male partner (a sort of sperm donor) is all that is needed to achieve pregnancy. However, clinical practice has shown that the quality of the male gamete is important for a successful ART outcome. Furthermore, the safety of ART has been questioned because of the high prevalence of comorbidities in the offspring of ART conceptions compared to spontaneous conceptions. These issues have paved the way for more research and a greater understanding of the mechanisms of spermatogenesis and male infertility. Consequently, numerous discoveries have been made in the field of andrology, ranging from genetics to several "omics" technologies, oxidative stress and sperm DNA fragmentation, the sixth edition of the WHO manual, artificial intelligence, management of azoospermia, fertility in cancers survivors, artificial testis, 3D printing, gene engineering, stem cells therapy for spermatogenesis, and reconstructive microsurgery and seminal microbiome. Nevertheless, as many cases of male infertility remain idiopathic, further studies are required to improve the clinical management of infertile males. A multidisciplinary strategy involving both clinicians and scientists in basic, translational, and clinical research is the core principle that will allow andrology to overcome its limits and reach further goals. This state-of-the-art article aims to present a historical review of andrology, and, particularly, male infertility, from its "Middle Ages" to its "Renaissance", a golden age of andrology.

Keywords: Andrology; Male infertility; Spermatozoa

INTRODUCTION

1. Male infertility: Dimension of the problem

Affecting nearly 186 million people worldwide [1], infertility has become a considerable public health issue [2]. The 2017 Global Burden of Disease study, which covers more than 195 countries between 1990 and 2017, reported a rate of increase in male infertility of 0.291% per year, with an upward trend in most countries (136/195) [2].

A male factor is a frequent cause of couple infertility affecting 2.5%–12% of men globally [3]. Several indicators of male reproductive health have deteriorated over the past 50 years. Indeed, a meta-analysis showed a significant decline in sperm count between 1973 and 2011, particularly in North America, Europe, Australia, and New Zealand (-5.33 million spermatozoa per year; -1.6% per year) corresponding to an overall decline of 59.3% [4]. In parallel, over the past 50 years, the prevalence of testicular tumors has increased, serum testosterone concentrations have decreased, and puberty disorders in recent birth cohorts have increased [5,6]. The environment may play an important role in impairing reproductive health alteration [5-7]. Another indicator of this growing infertility pandemic is the increased use of assisted reproductive technologies (ARTs), which rose from 2.4% to 18.3% annually in Europe, the United States, Australia, and New Zealand between 2012 and 2016, with an impressive 1.3 million ART cycles performed in 2016 in these countries [8].

The crucial question, especially in economically intensive industrial societies, is whether the trend of increasing infertility can still be reversed and whether limiting the harmful impact of the environment on reproductive capacity will be sufficient for the sustainability of the human species. This explains why it is urgent to implement more and more research into the causes of male infertility.

2. Epidemiology of male infertility

Infertility can be caused by a male factor, a female factor, a combination of them, or can be unexplained.

The male partner of the infertile couple is widely quoted as responsible, either alone or in association with a female factor, in about 50% of cases (reviewed by [3,9]). This is generally supported by studies from around the world, although the results of specific studies can be drastically different [10-17]. For example, the male infertility rates in these various studies were reported to range from 9.16% to 45.6%, while the combined male and female infertility factor rates were between 2% to 39%. Male factor infertility may be present in some cases of unexplained infertility, as demonstrated by ultrastructural abnormalities of the sperm centriole that could compromise sperm quality and function [18,19].

Despite this evidence, the male partner of the infertile couple seems to be neglected in most cases for a variety of reasons [20]. Especially in patriarchal societies, infertility is considered a "female health problem" and gynecologists play a key role in the management of both male and female infertility cases [3]. Even in developed countries, many ART clinics do not have a physician expert in andrology or a urologist skilled in male infertility within a 60-minute travel time radius [21]. In fact, the same study reported that 13 states in the United States have no male infertility specialists at all. This can be construed as an impact of the social constructs of society and its assigned gender roles in which women are considered as having the main role in conception and pregnancy while men are seen as secondary participants [22]. A North American survey of 4,335 males seeking infertility consultation found that the average age of male patients evaluated by an andrologist is 37 years, the majority (74%) of whom did not have a previous assessment of infertility. Among them, 6.4% and 10.7% of the infertile couples had already undergone intrauterine insemination and *in vitro* fertilization (IVF) respectively prior to seeking assessment for potential male factor infertility [23].

3. Male infertility and male general health

A recent meta-analysis reported that infertile males have a higher risk of mortality compared to their fertile counterparts. This risk increases in parallel with the severity of deterioration in sperm quality [24]. It is widely accepted that various medical conditions could pose harmful effects on spermatogenesis, and many studies suggest that male infertility serves as a biomarker of overall male health [25]. For example, a prospective cohort study with 899 infertile patients found that non-obstructive azoospermia (NOA) is an independent factor associated with a higher Charlson Comorbidity Index, a score used to predict 10-year survival in patients with multiple comorbidities [26].

Evidence also supports that male infertility has a bidirectional association with malignancies and chronic diseases [27]. It is perturbing to know that male infertility is considered a biomarker of individual and familial cancer risk. Indeed, infertile patients appear to have a higher risk of developing testicular tumors, prostate cancer, acute lymphoblastic leukemia, non-Hodgkin lymphoma, and thyroid cancer [28]. Similarly, infertile patients are more likely to develop testicular germ cell tumors (TGCT). In a study on 32, 442 Danish males who were assessed for infertility, the risk of developing TGCT was found to be 1.6 times higher (standardized incidence ratio 1.6, 95% confidence interval [CI] 1.3–1.9) than in the general population [29]. Furthermore, a recent meta-analysis found that patients with male infertility and testicular microcalcifications on ultrasound examination had an 18 times greater risk of developing testicular tumors [30].

Evidence also suggests that male infertility is associated with an increased risk of cardiovascular diseases. A recent population-based cohort study included 2,326 infertile patients, who were compared to 9,304 controls [31]. The results of this study showed that infertile patients have a higher risk of developing cardiovascular diseases. Furthermore, male infertility can also be linked to metabolic syndrome. The main mechanisms involved are oxidative stress, and metabolic, and hormonal alterations [32].

However, a recent analysis of the National Survey for Family Growth in the USA (2011–2017) comparing 6.5 million subfertile patients with 26 million fertile men, failed to demonstrate a significant association between infertility and rates of comorbidities [33].

4. Aim

Currently, andrology is experiencing a sort of "Renaissance". An increasing number of articles are being published and the comprehension of the molecular mechanisms underlying spermatogenesis is becoming clearer. Nevertheless, Andrology comes from the "Middle Ages", a period when it was very far from being understood and fully acknowledged; ages when the male partner of the infertile couple was often neglected. This historical review aims to depict the state

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of the art in Andrology, and, specifically, male infertility, from the "Middle Ages" to the "Renaissance".

THE MIDDLE AGE OF ANDROLOGY

Andrology was initially considered a division of urology, gynecology, endocrinology, and to some extent dermatology. It was first introduced by, Dr. Harald Siebke, a professor of gynecology, who argued that andrology should be the male counterpart of gynecology [34]. It took several years for the first scientific journal devoted to andrological sciences to be born. Indeed, Dr. Carl Schirren began publishing Andrologia in 1969 [35]. Subsequently, national societies or study groups in andrology were established [36] which, today, actively operate in this field.

However, the development of andrology was not without its obstacles. An example is the uncertainty about the definition of normal sperm parameters in conventional semen analysis. For decades, the diagnosis of oligozoospermia has been based on sperm concentrations of <20 million spermatozoa/mL in two semen analyses. In 2010, the World Health Organization (WHO) reevaluated its criteria using established cutoffs of the parameters of a population of fertile men, and oligozoospermia was diagnosed when sperm concentration was <15 million spermatozoa/mL [37]. This definition was further partitioned, depending on the number of spermatozoa, into mild (10–15 million/spermatozoa/mL), moderate (5–10 million spermatozoa/mL), and severe (<5 million spermatozoa/mL). However, in the latest sixth WHO manual, the editors advocate the use of multiple criteria to establish a diagnosis of male infertility instead of using sole reference values [38].

In the early years, genetic knowledge was minimal and confined to chromosome abnormalities. By the 1920s, the human karyotype had been described as 46 chromosomes [39]. It took another 30 years before the diploid human chromosome was described as 22 pairs of autosomal chromosomes and 1 pair of heterochromosomes, *i.e.*, XY for men [40,41]. For this reason, many clinical decisions relied on simple laboratory analyses without standardization in various andrological fields [42-44].

The advent of ARTs in the 1980s allowed millions of infertile couples around the world to conceive. Intracytoplasmic sperm injection (ICSI), introduced in the 1990s, revolutionized the treatment of infertility since the common belief was that a spermatozoon and an egg were enough to initiate a pregnancy. Since then, ICSI has been broadly suggested to couples, even without being preceded by any attempt to identify and treat the etiological factors responsible for couple infertility [45]. In fact, ART is thought to mainly treat the female partner [46]. The fertility status of the male partner is, in contrast, often overlooked and his involvement was limited to only obtaining and manipulating his gametes [47].

With the introduction of ICSI, there has been a paradigm shift in andrology that has led to the motto "find and select a spermatozoon to achieve a pregnancy" instead of improving the fundamental understanding of why men are infertile, the contribution of abnormal sperm components to male infertility, and the treatment that can be put in place to overcome infertility [46]. Moreover, advances in ART have opted to focus on female infertility and improving ART outcomes leading to a large gender discrepancy in reproductive research, which consequently slows the progress of research in andrology.

However, ART is not a panacea for all fertility problems. Recent studies suggest physicians should have a comprehensive clinical, molecular, and cytogenetic approach to infertility in addition to addressing general factors, such as lifestyle changes and the effects of environmental pollution, which have a great impact on male fertility. Given the cost and burden that comes with using invasive technology in ART, understanding the etiology of male infertility more accurately is essential for the fertility specialist to overcome inefficiency or any unproductive step in the process of fertilization as well as to appropriately advice patients on their chances of success with the use of ARTs [48].

Despite the increase in ART-related pregnancies, the safety of these techniques continues to be a matter of concern. Children born *via* ARTs are at increased risk of significant congenital disabilities compared with children born through natural conception [49]. A retrospective study on 59,971 couples from the Japanese ART registry whose female partners received fresh embryo transfer from 2007 to 2014 suggests that underlying male subfertility may play a role in the risk of significant congenital disabilities related to ICSI and IVF [50]. Recent studies showed that children born through ICSI had significantly increased risks of urogenital anomalies (odds ratio=1.27, 95% CI 1.02–1.59) [51]

and specific major congenital cardiac birth defects [52] compared with children born after IVF. However, these results should be cautiously interpreted since it is not certain whether the increased risk of major comorbidities in children born after ICSI is due to the technique itself or infertility [53].

THE RENAISSANCE OF ANDROLOGY

Conventional semen analysis has long remained the only routine diagnostic test for male infertility, despite the lack of capability in discriminating fertile men from infertile patients [54]. There is now a demand to explore additional diagnostic tools to facilitate the prediction of fertility and direct management options of couples with male factor infertility. The Renaissance, which marked the transition from the Middle Ages to modernity during the 15th and 16th centuries, is characterized by the efforts to surpass the ideas and achievements of classical antiquity. Likewise, modern andrology is currently experiencing its own "Renaissance". This is in effect a golden age of andrology, which involves many areas of the andrological sciences (Fig. 1) [44,55-72].

1. Genetic testing

Genetic factors are accountable for up to 15% of men with infertility, with XXY or Klinefelter syndrome being the most detected genetic abnormality [73]. Karyotyping, Y-chromosome microdeletion assays, and the evaluation of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations represent the traditionally available genetic tests that are offered to infertile patients [74]. These genetic analyses are nowadays routinely requested but have not changed much in the last 20 years. Despite a careful diagnostic workup that includes genetic testing, the reason for infertility remains elusive in up to 70% of cases [75]. In recent years, dozens of articles on the genetics of male infertility have been published, with an aim of identifying more accurate genetic tests in the attempt to increase the diagnostic yield in patients with severe male factor infertility such as azoospermia [75-79].

The latest edition of the WHO Laboratory Manual of Semen Analysis has introduced, for the first time, the assessment of sperm aneuploidy and sperm DNA fragmentation (SDF) rates, indicating these genetic tests as an extended examination [80]. However, many other genes play a vital role in reproductive function. The genetics of male infertility is highly complex and heterogeneous as shown by a list of at least 2,300 genes called into play in spermatogenesis [81]. It is therefore not surprising that the current tests used are not robust enough to pinpoint a specific diagnosis in many cases and the etiology of infertility remains unknown in most infertile patients [82].

With the recent advances in reproductive medicine, the future of genetic testing is promising with methods

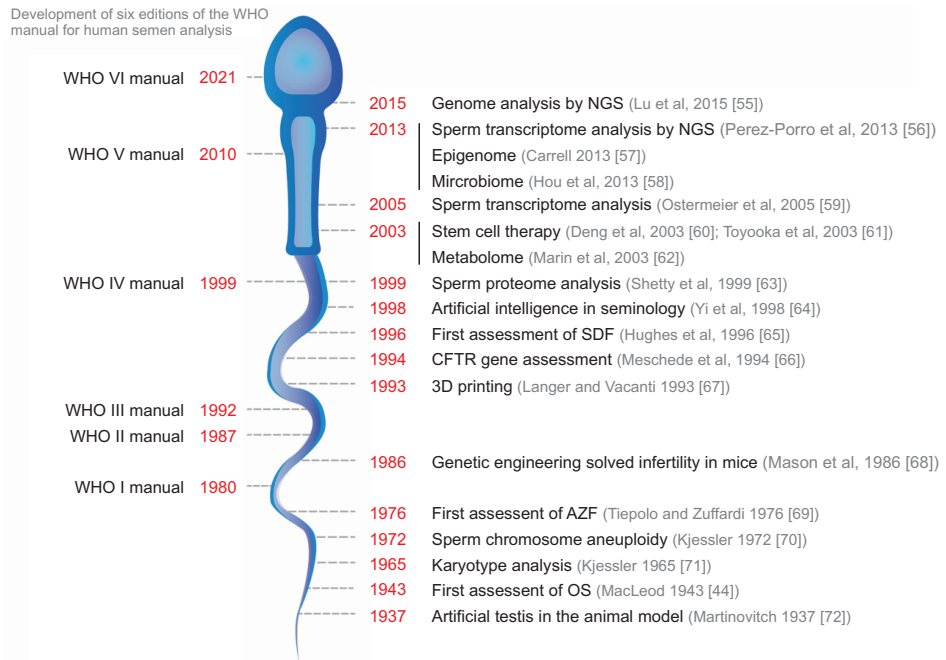

Fig. 1. Notable/major discoveries in the field of male infertility. Several significant discoveries have taken place in the andrological field in the last 85 years. They consist of genetics, several "omics" technologies, oxidative stress and sperm DNA fragmentation, the sixth edition of the WHO manual for semen analysis, artificial intelligence, management of azoospermia, fertility in cancer survivors, artificial testis, 3D printing, gene engineering, stem cells therapy for spermatogenesis, reconstructive microsurgery, and seminal microbiome. AZF: azoospermia factor, *CFTR*: cystic fibrosis transmembrane conductance regulator, NGS: next-generation sequencing, OS: oxidative stress, SDF: sperm DNA fragmentation, WHO: World Health Organization [44,55-72].

capable of characterizing the genome, such as microarray and next-generation sequencing (NGS) [83]. NGS includes potential tests, such as disease-targeted sequencing, whole-exome sequencing, and whole genome sequencing [84]. These tests can analyze many genes simultaneously rather than one at a time [85]. This provides the ability to screen for multiple genetic issues including single nucleotide variants, copy number variations, and chromosome rearrangements. Furthermore, through the use of a custom-made gene panel, ongoing research is also trying to associate specific gene mutations with testicular histology and find the predictive value of some mutations on sperm recovery in patients with azoospermia [86]. Ongoing research intends to identify genetic abnormalities in areas such as chromosomal regions enriched in genes contributing to male infertility, genes shared among phenotypes, and the strength of association [79,87,88]. It is also hoped that the use of artificial intelligence (AI) with machine learning combined with improved genetic testing will make the genetic assessment more efficient and more cost-effective [89].

With the limitations of current genetic diagnostic tests, efforts should be targeted towards developing novel clinical assays to increase the identification of genetic etiologies that would decrease the prevalence of infertile patients diagnosed as idiopathic [90].

2. "Omics"

The "omics" technology has developed widely, and this has led to its application in male infertility. It includes genomics, transcriptomics, proteomics, epigenomics, and metabolomics.

RNA sequencing enabled the identification of thousands of RNAs in human spermatozoa [91,92]. Some of them are said to arise from spermatogenesis, others from the epididymis and passed to spermatozoa *via* the extracellular vesicles. However, a minority of RNAs could be transcribed *de novo* in mature spermatozoa. This is a revolutionary concept as it was believed that compact sperm DNA could not be transcribed [92]. The sperm RNA content of fertile men and infertile patients appears to be different. This underlines the future role of sperm RNAs in the diagnosis of male infertility [92]. Intriguingly, sperm RNAs can also be involved in transgenerational inheritance [92].

In an effort to identify the molecular targets of idiopathic male infertility (IMI), a systematic review comprehensively analyzed data coming from seminal plasma transcriptome and proteome of patients with IMI. The authors reported that specific microRNAs (miRNA) (miR34, miR-122, and miR-509) are downregulated in patients with NOA or oligozoospermia compared to fertile controls. It is currently unknown whether these targets have a predictive role in the outcomes of testicular sperm extraction (TESE) or ART [93].

According to proteomic studies, specific proteins (*e.g.*, ECM1, TEX101, LGALS3BP) in seminal plasma appear to be accurate predictors of TESE outcome. Furthermore, ECM1 can also predict ART success, being differentially expressed in patients with different ART outcomes [93]. This knowledge has a practical clinical value and, if further confirmed by a high-quality line of evidence, may play a role in future counseling and decision-making for infertile patients.

Epigenetic studies have been conducted in infertile men for the past thirty years. Recently, the accumulated knowledge highlighted the presence of an abnormal methylation pattern in the spermatozoa of infertile patients. Indeed, a meta-analysis reported different methylation of the *H19*, *MEST*, and *SNRPN* genes in infertile male patients compared to fertile controls [94]. The sperm methylation pattern has been suggested to influence ART outcomes. In particular, low methylation of *H19* appears to occur in patients with recurrent pregnancy loss (RPL), while altered methylation of the *GTL2* gene has been found to correlate with a poorer ART outcome [95].

Spermatozoa also contain cytoplasmic structures that function in the early embryo, such as centrioles. The spermatozoa were thought to have a single barrel-shaped canonical centriole, *i.e.*, the proximal centriole, which gives rise to the centrosome of the zygote. However, spermatozoa have recently been found to have an atypical second distal centriole that forms the second centrosome of the zygote [96,97]. The two sperm centrosomes bring together the male and female pronuclei and polarize their DNA, which is important for normal embryo development [98]. Centriole dysfunction is associated with unexplained male infertility [18,99,100].

Finally, emerging data indicate that metabolomics may also add some interesting insights into the diagnosis of IMI patients as it can explore the concentration of energy-related metabolites in seminal plasma. A recent study identified 21 compounds in the seminal plasma as biomarkers of male infertility [101]. Using

the Biomarker Score, an index that cumulates the biochemical characteristics of seminal plasma abnormalities, the authors found a significantly different score between fertile men and infertile patients.

3. Oxidative stress and sperm DNA fragmentation

The significant role of oxidative stress (OS) in the pathogenesis of male infertility has been supported by several authors during the recent years, leading to the introduction of a new concept of "male oxidative stress infertility" (MOSI) [102]. MOSI can explain many cases of male infertility that were previously described as idiopathic. Seminal reactive oxygen species (ROS), at small physiological amounts, are necessary for sperm hyperactivation and capacitation. However, excessive ROS production, exceeding the seminal fluid scavenging capacity, causes OS. As a result, ROS attack the polyunsaturated fatty acids (PUFAs) on the sperm plasma membrane, leading to the formation of reactive aldehydes (including malondialdehyde) that cause mitochondrial dysfunction with further production of ROS and further altered sperm function [102].

SDF includes mismatch of bases, loss of bases, base modifications, DNA adducts and crosslinks, pyrimidine dimers, and single- and double-stranded DNA breaks [103]. The last two decades have witnessed a growing interest in the influence of SDF on male fertility. Several meta-analyses have already confirmed the importance of the SDF in understanding the etiology of male infertility [104-110]. The evidence is of a high level; hence the evaluation of the SDF rate has been included in the latest sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen testing as an "Extended Semen Examination" [80]. To date, several methods are available to measure SDF, including the Comet assay, terminal deoxynucleotidyl transferase dUTP nick end labeling assay, sperm chromatin structure assay, and sperm chromatin dispersion test. However, which of these assays is the best and which are the cut-offs for predicting spontaneous or ART pregnancy remains to be clarified [103].

SDF is not only useful for understanding the etiology of male infertility but also for decision-making in patients with certain diseases. For example, the European Association of Urology (EAU) guideline [111] states that varicocele repair may be considered in patients with elevated SDF rates and otherwise unexplained male infertility, who have failed ART, including RPL, and embryogenesis or implantation failure. Varicocele repair can ameliorate SDF [112].

Currently, there is no firm conclusion as to the impact of high SDF on ART outcomes due to the great heterogeneity of the studies exploring the topic. Similarly, the use of testicular sperm to improve ICSI outcomes in patients with elevated SDF has been a matter of debate. According to a meta-analysis, the use of testicular sperm in patients with high SDF results in a significant increase in clinical pregnancy and live birth rates, and a decrease in miscarriage rates [113]. However, the evidence for the use of testicular sperm for ICSI in patients with high SDF is of poor quality [114]. Additionally, no SDF test has been standardized for the use of testicular sperm.

4. WHO manuals

Since the 1980s, six versions of the WHO manual for semen analysis have been published, with the 6th Edition being recently published in 2021. The latter is another novelty that characterizes the Renaissance of Andrology. Table 1 shows the reference values for semen characteristics as depicted in the various editions of the manual.

The latest manual incorporates changes governing the clinical andrological practice for the next ten years [80]. It also includes many laboratory-related updates, including the different methods related to quality control and assurance [115]. The main change that is considered as a real novelty is the abandonment of the thresholds used in the 5th Edition, stating that the 5th percentile previously used as the lower limit is not an adequate tool to distinguish fertile men from infertile patients and thereby suggesting a more holistic approach in the assessment of fertility. The sixth edition instead introduced *"decision limits"* and assumed that an optimal assessment of male fertility should be based on a combination of the information gathered from the semen analysis and other available clinical parameters. Furthermore, the WHO 6th edition confirmed the significance of sperm DNA integrity with a demonstration of the different techniques, while acknowledging the role of the SDF test within the context of male infertility [38].

Despite these positive changes, the WHO 6th edition is still lacking in several respects. For example, data on fertile men from South America and sub-Saharan

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Table 1. Comparison of semen characteristics in the World Health Organization (WHO) manuals for semen analysis

Semen parameters	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010	WHO 2021[a]
Volume (mL)	ND	≥2	≥2	≥2	≥1.5	≥1.4
Sperm concentration (10^6/mL)	20–200	≥20	≥20	≥20	≥15	≥16
Total sperm count (10^6)	ND	≥40	≥40	≥40	≥39	≥39
Total motility (%)	≥60	≥50	≥50	≥50	≥40	≥42
Progressive motility	≥2	≥25%	≥25% (a)	≥25% (a)	≥32% (a+b)	≥30% (a+b)
Sperm vitality (%)	ND	≥50	≥75	≥75	≥58	≥54
Normal morphology (%)	80.5	≥50	≥30	14	≥4	≥4
Leukocyte concentration (10^6/mL)	<4.7	<1.0	<1.0	<1.0	<1.0	ND

ND: not defined, a: type a progressive motility, b: type b progressive motility.
[a]The WHO 2021 edition removed any thresholds but kept decision limits. These are the 5th percentile of semen examination results from 3,500 men in 12 countries of couples starting a pregnancy within one year of unprotected sexual intercourse leading to a natural conception.

Africa are sparsely represented. There is also a lack of clear reference thresholds to be applied in place of the percentiles of the basic seminal parameters of the fifth edition. This could hamper the physician's ability to make a correct assessment, as the decision limits have not yet been clarified in practice. Therefore, professionals around the world may prefer to continue using the fifth edition reference values instead of the current edition. The more detailed sixth edition manual also runs the risk of being too technical, which could delay its widespread use among clinicians. Although SDF testing was recognized in this latest edition as having a role in the evaluation of the infertile patient, in the sixth edition neither the threshold (cut-off) values nor the indications for the SDF test and the criteria to interpret the test results were described. Furthermore, advanced tests for seminal OS appeared to be more for research purposes than for actual clinical use [38].

5. Artificial intelligence

During the past few years, AI (using machine learning-based systems) has heavily influenced innovations and research in ART. AI may assist or even fully automate ART procedures, such as gamete quality assessment, sperm selection for ICSI, oocyte collection, assisting with protocols for controlled ovarian hyperstimulation, donor matching, or selecting and ranking embryos for transfer and cryopreservation [116]. Furthermore, AI may help optimize and standardize clinical processes by introducing predictive maintenance in ART instruments and automatically extracting and analyzing key performance indicators to carry out continuous quality control [117]. Conversely, selecting the best gamete is not necessarily the best solution, even if advances are made in AI and automatic selection. While AI offers great strengths and opportunities, it also comes with several weaknesses and threats [118].

Another field of andrology where AI may play a role is seminology, as AI can overcome the subjectivity of semen analysis evaluation. Most AI studies have focused on morphology assessment. Some studies have developed an AI model that evaluates only the morphology of sperm heads, while others have created a model evaluating the whole spermatozoon. These differences make it difficult to compare results and find useful implications for clinical practice [119]. Some reports also support the role of AI in assessing sperm concentration, progressive sperm motility, and total motile sperm count [120]. Despite the promising implication and capability of AI, more challenges need to be resolved prior to more extensive application in clinical practice [119].

A future challenge is the development of AI models for ultrasound evaluation of the male genital tract (testes, epididymis, vas deferens, prostate, ejaculatory ducts, and seminal vesicles), and for the diagnosis of male accessory gland infection (MAGI) [121,122]. AI is used in magnetic resonance imaging (MRI) for the diagnosis of prostate cancer [123]. At the same time, the interest in the use of AI in MRI of testicular tumor patients is growing [124]. There is currently no application for AI in andrological ultrasound, although specific ultrasound criteria have been identified for the diagnosis of MAGI [121,122].

6. Management of azoospermia

The management of azoospermia remains a challenging situation. However, the sperm recovery rate is

improving with the development of the micro testicular sperm extraction (mTESE) technique, as well as the outcomes of ARTs [125]. A meta-analysis showed that the outcome of ICSI is not affected by whether spermatozoa retrieved from the testis are fresh or frozen [125]. Another meta-analysis showed that ICSI outcome in cryptozoospermia is superior when testicular spermatozoa are used compared to ejaculated ones [126]. The reason for this finding is related to the OS to which spermatozoa are exposed during epididymal transit, which can be avoided by the use of testicular sperm, which results in better embryo quality and higher implantation and pregnancy rates. Understanding that IVF/ICSI outcomes using fresh *versus* frozen spermatozoa are equivalent in patients with NOA, undergoing sperm recovery *via* mTESE allows them to proceed independently from the female partner and any type of IVF cycle.

The role of OS and the role of antioxidants in infertility has been gaining significance. This is partially due to reports of functional polymorphisms of genes such as nuclear factor erythroid 2–related factor 2 (*NRF2*), superoxide dismutase (*SOD*), and other candidate genes [127]. Even in men with obstructive azoospermia, reports of antioxidants improving the fertility potential of sperm, especially after failed first IVF cycle, have attributed a role to OS associated with stasis in the pathogenesis of infertility [128]. The specific role of antioxidants in azoospermia or cryptozoospermia is yet to be defined.

7. Fertility in cancer survivors and artificial testis

Urologists and reproductive specialists dealing with male infertility are the main health professionals who treat men interested in fertility preservation/restoration following a newly diagnosed cancer or after completion of their oncological treatment. It is imperative that physicians discuss the risk of infertility with oncology patients receiving gonadotoxic treatment during their reproductive years or with the parents/guardians of children who may be infertile in the future because of their cancer treatment. Healthcare professionals should motivate the patients about fertility preservation options and/or refer them to specialists in reproductive medicine. A documented discussion on sperm, oocyte, and/or embryo cryopreservation is standard practice [129].

Despite many articles, guidelines, and position statements on fertility preservation and its necessity, access to fertility preservation is still insufficient. The French Cancer Cohort covers the entire French population and includes approximately 7 million cancer patients. A study based on this cohort, which excluded patients treated only surgically for cancers in locations distant from reproductive medicine centers or being treated for cancer in the last 3 years, identified 10,392 men under 50 years. Of them, only 5,640 men (54.3%) received fertility preservation for any reason (including non-oncological etiologies) in 2015 [130].

Another cohort study from Norway analyzed the reproductive and marital status of male cancer survivors younger than 25 years of age and cancer-free male controls. Men who survived cancer (n=2,687) had reduced paternity (hazard ratio [HR]: 0.72, 95% CI: 0.68–0.76) and were more likely to undergo ART (risk ratio: 3.32, 95% CI: 2.68–4.11). In addition, brain cancer survivors were less likely to get married (HR: 0.93, 95% CI: 0.86–1.00) [131].

Huyghe and colleagues [132] reported that nearly a third of the patients (both men and women) younger than 50 years would have preferred a fertility consultation before cancer treatment and 24% of men would want to visit a reproductive health clinic in the following year. In addition, the authors concluded that it would be ideal to build a multidisciplinary reproductive health clinic as part of a tertiary care cancer center [132].

8. 3D printing

Faced with the need to extend fertility preservation in cancer survivors, molecular biology is actively involved in the development of techniques that can help preserve fertility, especially when immature germ cells can be retrieved. One example is the artificial testis with three-dimensional (3D) printed scaffolds.

Three-dimensional scaffolds are believed to act as a key factor in cellular interactions and may play the role of the extracellular matrix (ECM) for *in vitro* culture and maturation [133]. Several studies have shown that ECM scaffolds can be used to produce a functioning artificial organ by combining stem cell culture [134]. Cell culture on ECM creates a normal tissue-like environment to allow differentiation of spermatogonial stem cells (SSC) *in vitro* [135]. Baert and colleagues demonstrated that scaffolds of pieces of testicular tissue

enhanced SSC proliferation [136]. Studies in pigs [137], and mice [138] have showed that the use of decellularized testicular scaffolds improves cell organization and serves as a valuable tool for studying the development of seminiferous tubules. Therefore, the testicular ECM can serve as a suitable biomaterial for SSC culture.

The printed scaffolds create controlled and precise 3D shapes. Therefore, a high-resolution and biocompatible tissue or material should be used for 3D printing [139]. Biomaterials used for printed scaffolds usually include hydrogels based on natural (collagen, gelatin, alginate, agarose, hyaluronic acid, and fibrin) or synthetic polymers [140]. Studies in this area are still ongoing.

Recently, Canadian researchers successfully bioprinted human testicular cells, which produced potentially viable spermatozoa [141]. Human testicular cells were derived from a single donor with NOA and Sertoli-cell-only syndrome, which was then bioprinted. After twelve days of observation, Sertoli, Leydig, peritubular myoid, and meiotic germ cells were present upregulating the expression of spermatogenic genes. This research shows that bioprinting results in high testicular cell viability without loss of the major somatic phenotypes in the testicular tissue. This breakthrough is a game-changer for patients with NOA [141]. However, this advancement still requires in-depth future research, both from a medical and ethical point of view.

9. Genetic engineering: CRISPR/Cas9 and non-obstructive azoospermia

The CRISPR/Cas9 system has the potential to radically transform reproductive medicine when considering gene repair. CRISPR/Cas9 method allows modifying nucleic acids, which make up the genome of all living organisms. The CRISPR/Cas9 system can be used for not only rapidly generating knockout mice, but also for more complicated gene manipulations. Through the mechanism of cutting and sewing the genome, it will be possible to identify the defective DNA locus and replace it with a functioning sequence leading to the restoration of the gene functions.

Using this technology, the functions of 30 testis-enriched genes and 4 ubiquitously expressed genes in male reproduction were analyzed by generating knockout mouse lines using the CRISPR/Cas9 enzyme. Knockout males showed normal fertility, suggesting that these 34 genes are dispensable for male fertility. According to the authors, disseminating this information to the scientific community is of pivotal importance to avoid unnecessary expenditure of time and research funds on these targets [142].

More interestingly, Li et al [143] choose the Kitw/Kitwv mouse as their research model to better develop gene editing in NOA. After the isolation of mutant SSCs, the authors performed the C-to-T point mutation at the Kitwv site of these SSCs and then performed the correction through CRISPR-Cas9-mediated homology-directed repair *in vitro*. The repaired SSCs were screened out, proliferated, and transplanted into the testis, and a complete spermatogenesis cycle was established in the recipient testis [143].

Obviously, this treatment strategy could be feasible only in patients who still exhibit SSCs since it is crucial to their isolation from the testis. In fact, by the isolation of SSCs and further gene editing, it could be possible to transplant to the patients with NOA and study the regeneration of spermatogenesis.

10. Stem cell therapy and spermatogenesis

Despite progress in ART, many couples are still unable to have healthy babies. Stem cells have brought new hope in overcoming issues related to infertility, which take advantage of cell-based therapies in both preclinical and clinical models. These cells are undifferentiated and, in adult tissues, can self-renew and multi-directionally differentiate when required [144]. According to their origin, stem cells are classified in humans as below:

Embryonic stem cells (*ESC*) have the indefinite ability to self-renew, differentiate into lineages (ectoderm, endoderm, and mesoderm), and maintain the normal karyotype during growth.

Induced pluripotent stem cells (*iPSC*) originate from ESCs as demonstrated by their morphology, surface markers expression, telomerase activity, and differentiation capacity in all three lineages, and can maintain a normal karyotype during growth in a way that is superior to ESCs due to their origin from adult cells. The use of iPSC allows overcoming the ethical issues related to the use of embryonic stem cells. Moreover, by developing from the somatic cells of the same patient, the chances of rejection are lower.

Mesenchymal stem cells (*MSCs*) have high proliferative potential and adhesion capacity, symmetric and asymmetric division, fibroblast-like morphology, easily induced differentiation, and the ability to form colonies

in culture. MSCs are considered an ideal cell type to treat patients with azoospermia due to their involvement in processes such as cell survival, proliferation, migration, angiogenesis, and immune modulation [145].

SSCs play a crucial role in maintaining the complex, highly productive process known as spermatogenesis through self-renewal and unlimited differentiation into spermatogonia, followed by the formation of haploid spermatozoa. SSCs are not widely used in regenerative medicine due to their low testicular number and difficulty in identifying them for successful isolation and culture [144].

The clinical translation of stem cell therapy in reproductive medicine is restricted due to existing problems and difficulties. These include unwanted differentiation and oncogenicity of iPSC and the chance of MSCs propagating tumor growth. In clinical practice, advances in stem cell therapy require further long-term planning under rigorous evaluation and supervision to ensure accuracy, quality, and safety. As autologous stem cells are more ethical, safe, and non-immune, their clinical application in the future holds better potential [146].

11. Surgical breakthroughs

Advances in technology in the laboratory and the operating room has greatly improved the use of surgery in cases of male infertility for diagnosis, increase sperm production or delivery, or sperm retrieval [147]. The essential technique in male infertility surgery would be microsurgery. Since vasovasostomy was introduced by Drs. Owen and Silber in the 1970s, microsurgery has become the standard procedure in male reproductive microsurgery [148].

Varicocele is the most prevalent surgically correctable cause of male infertility [149]. Current evidence suggests that microscopic approaches may have a lower recurrence and complication rate than non-microscopic ones in varicocele repair [150]. The benefit of varicocele repair includes the improvement of sperm quality and higher rates of sperm retrieval and pregnancy, even in patients with NOA [151,152]. On the other hand, patients with obstructive azoospermia, especially post-vasectomy, may have an increased pregnancy rate from the refined surgical technique used for vasovasostomy and vasoepididymostomy [153,154]. As a last resort, sperm retrieval can be chosen for more challenging cases to find spermatozoa to use for ART [155].

Several technological steps have been developed to improve the surgical procedure, such as 3D video microscope surgery, robotic-assisted surgery, and multiphoton microscopy [156]. Video microscope surgery uses a 3D camera and offers optimal vision and ergonomics [157]. Finally, multiphoton microscopy is an auxiliary technology tool that uses non-linear excitation for fluorescence intended for better viewing during mTESE [156]. However, it has not been well studied in humans, with only one pilot study conducted on *ex vivo* human testis histology [158].

Despite new technological advantages, the cost for both initial investment and maintenance should always be considered before its use. Cost-efficacy for robotic-assisted and 3D video microsurgery is still not clear and requires further research [156]. From this point, we still look forward to the unlimited possibility of future breakthroughs in surgery, such as the real-time adaptation of AI that may assist and improve the outcomes of male infertility surgeries [156].

1) The use of the da Vinci robotic platform for microsurgical vasectomy reversal

Robot-assisted vasectomy reversal (RAVR) may replace the conventional operative microscope offering technical advantages including improved stability, decreased microsurgeon fatigue by improving ergonomics, eliminating physiologic tremor, high-definition three-dimensional optics, scalability of motion, surgeon control of instrumentation including a camera and 3 microsurgical instruments simultaneously, foregoing the need for a specialty skilled microsurgical assistant, and improving operative times. For optimal outcomes, a high level of training and skill in microsurgical techniques is crucial [157].

In 2004, the first publication on robot-assisted vasovasostomy *ex vivo* demonstrated the elimination of physiological tremor and comparable patency rate [159]. This was followed up with RAVV and robot-assisted vasoepididymostomy (RAVE) in a rat model demonstrating improved stability and motion reduction during suturing [160]. A rabbit model was then utilized to demonstrate a multilayered RAVV technique [161]. Parekattil et al [162] published the first human study on RAVR demonstrating the robotics ability to shorten operative times (109 minutes *vs.* 128 minutes) and a higher mean sperm counts (54 *vs.* 11 million) post-operatively compared to microsurgery. This was followed

by a human validation study that compared conventional microsurgery, revealing equivalent operative times, patency rates, sperm concentrations and total motile sperm count, with a mean faster time to pregnancy by 4 months in the robotic group [163]. A learning curve study revealed that 75 RAVVs are necessary for a novice robotic microsurgeon to achieve equivalent operative and anastomosis times compared to conventional microsurgery [164]. Although the pros and cons of the application of the robotic platform to vasectomy reversal have been debated, the use of this technology has gained traction with more reproductive urologists implementing its use [165].

12. Seminal microbiome

The testis and semen are not sterile and contain significant quantities of a distinct microbiome that is rich in both fertile and infertile men [166,167]. The changes in its bacterial taxa composition (*e.g.*, *Ureaplasma urealyticum*, *Anaerococcus*, *Enterococcus faecalis*, *Mycoplasma hominis*, and *Prevotella*) are associated with changes in semen quality, sperm function, and fertility [168]. The seminal microbiome has essential implications for the male's reproductive health, the couple's health, and even the offspring's health, because of the transfer of microorganisms to the partner and offspring [169]. It has been indicated that manipulating the human microbiome may be effective in improving semen parameters, sperm quality, and fertility outcomes [169].

CONCLUSIONS

This review highlights current challenges, recent advances, and the latest findings in the management of male infertility. Coming from the "Middle Ages", andrology is currently experiencing its "Renaissance", a sort of golden age. Indeed, current evidence is shedding light on previously unknown issues. The numerous discoveries made in recent years in andrology range from genetics to "omics" technologies, OS, SDF, WHO manuals, AI, management of azoospermia, new fertility preservation techniques, gene engineering, stem cell therapy, and surgery. However, as many cases of male infertility remain diagnosed as idiopathic or unexplained, more research is needed to uncover the mechanisms whose understanding will have a major impact on the clinical management of infertile male patients. A multidisciplinary approach that includes clinicians and scientists working together on basic, translational, and clinical studies is the fundamental principle for the further advancement of andrology.

Conflict of Interest

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