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Review

# Evolution of the incidence of male fertility disorders over the last decades

Francesco Pallotti [a], Alessandra Buonacquisto [a,b], Gaia Cicolani [b], Anna Chiara Conflitti [b], Francesco Lombardo [b], Donatella Paoli [b,*]

[a] Department of Medicine and Surgery, "Kore" University of Enna, C.da Panasia, 94100, Enna, Italy

[b] Laboratory of Seminology - Sperm Bank "Loredana Gandini", Department of Experimental Medicine, "Sapienza" University of Rome, Viale del Policlinico 155, 00161, Roma, Italy

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ABSTRACT

Male reproductive health evaluation involves andrological and sexological clinical problems but, on a wider scale, it also involves other public health issues, including lifestyle and metabolic factors. These factors, especially if combined with pre-existing andrological diseases, have been found to be significantly associated with semen parameters impairment and, thus, male infertility. A hypothesized trend of decline of male fertility has been debated in the last years and this aspect is strictly intertwined with the evolution of the incidence of male fertility disorders over the last decades. It should be stressed that all these factors harbour relevant repercussions for both individual reproductive health as well as broader socio-economic and demographic issues. Thus, the aim of this review is to perform a short and critical overview of the andrological issues affecting the reproductive outcomes of the infertile male as well as provide a comprehensive and critical overview of the male fertility decline issue.

## 1. Introduction

Male reproductive health can be considered an important and complex healthcare issue. Its thorough evaluation involves andrological and sexological clinical problems but, on a wider scale, it also involves other public health issues, ranging from sexually transmitted infections (STIs), declining fertility and testicular cancer, all reported to have an increased incidence in the last years. Human fertility is reduced compared to other mammalian species and it can be stated that a condition of hypofertility is physiological in the sense that a certain waiting period is required before the chance to conceive becomes significant. In this sense, fertility can be considered a probabilistic biological event. Thus, infertility is defined as the inability to conceive after 1 year of unprotected intercourse and is becoming a major burden worldwide. Estimating the percentage of infertile couples in the world is particularly problematic, also considering the inevitable difficulties in quantifying new couples formed each year outside of marriage. Despite these limitations, there is a general agreement that this problem involves about 10–20% of couples in industrialized countries. Furthermore, a definite cause often cannot be identified in over one third of cases. Another important consideration is that the fertility status often does not concern the individual partner but results from the interaction of the health status (both general and reproductive) of the two partners. Therefore, it is generally more correct to use the term "couple infertility", including both male and female factors. It is also necessary to distinguish between primary infertility, a term used for couples who have never conceived and secondary infertility, when a full-term pregnancy is noted in the medical history with either the current or a previous partner. There are various pathophysiological endocrinological and andrological causes for the male factor infertility and also general health problems could influence male fertility status (Pallotti et al., 2022; Ferlin et al., 2022; Ferlin et al., 2021). Furthermore, the real-life impact of male infertility could be underestimated due to the heterogeneity of its clinical approach: too often, the diagnostic process is limited to semen analysis and, likewise, the treatment phase is hastened by addressing the couple directly to assisted reproduction techniques (ART) (Ferlin et al., 2022). The very definition of "idiopathic" or "unexplained" infertility should be restricted in its use only in cases where a thorough diagnostic procedure has been carried out. In general, aside from medical conditions like varicocele and other well-defined congenital or acquired causes of infertility, lifestyle factors

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* Corresponding author. Laboratory of Seminology - Sperm Bank "Loredana Gandini", Department of Experimental Medicine, "Sapienza" University of Rome, Viale del Policlinico 155, 00161, Roma, Italy.

*E-mail address:* donatella.paoli@uniroma1.it (D. Paoli).

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ranging from eating habits to smoking and alcohol consumption may impact significantly on male fertility (Salas-Huetos et al., 2017; Petre et al., 2023) as well as environmental/occupational exposure to pollutants and endocrine disruptors (Cargnelutti et al., 2021, 2022) and metabolic conditions (Venigalla et al., 2023). These factors, especially if combined with pre-existing andrological diseases, have been found to be significantly associated with semen parameters impairment and, thus, male infertility (Cargnelutti et al., 2023). In current reproductive medicine practice, both andrologists and gynecologists have the pivotal role of directing the decisional diagnostic and therapeutic network, carefully balancing the couples' needs and reproductive medical options. The increasing awareness of andrological diseases and the increasing level of care in the last decades, may lead to discovery of both "common" andrological diseases and metabolic comorbidities that may synergistically hinder the couple's reproductive chance. Even though medically assisted reproductive procedures have the potential to overcome these diseases, overlooking to diagnose conditions potentially affecting male fertility is invariably associated with an increased risk of negative reproductive outcome, increased number of ART procedures and, thus, increased costs and a non-negligible psychological burden for the couple itself. Thus, the aim of this review is to perform a short and critical overview of the andrological issues affecting the reproductive outcomes of the infertile male.

## 2. Demographic trends

Considering data from the Italian context, the National Institute of Statistics (ISTAT) estimates that every year in Italy there are at least 30–45 thousand new couples experiencing reproductive issues. According to the ISTAT data (year 2021) in 2021, births registered an 11% decrease compared to the previous year (−4643). The declining birth rates correspond to the low mean number of children per Italian woman (~1.25). Moreover, this decrease is almost entirely attributable to births from couples where both parents are Italian (314,371 in 2021, almost 166 thousand less than in 2008). A growing phenomenon, however, is the use of Medically Assisted Reproduction (MAR) techniques, which is carried out on average in 2.9 pregnancies out of 100 (ISTAT, 2021; http://dati.istat.it/). Human reproduction is the result of the action of numerous biological and behavioral factors, which are in turn influenced by the social, economic, and institutional context. This means that apart from known pathological conditions linked to couple infertility, also non-biological factors may further interfere and, in some cases, may have an even more significant negative impact on fertility, including economic and social issues, which may force the couple to postpone pregnancy planning. Demographic data clearly remark that the average age at which a couple seeks a first pregnancy has been steadily increasing over the years, and this trend is likely to recognize at least in part socio-economic factors, including salaries, institutions and work-family reconciliation policies (nurseries, working hours, etc.) that may further modulate the couple's family planning irrespective of reproductive health issues. Yet these factors, if corrected, could have a direct effect on the fertility levels of a population, meaning that their variation could reasonably produce a change in the number of conceptions and births or in a woman's ability to have children.

## 3. Andrological diseases and lifestyle behaviors

Regarding "organic" diseases, among the commonest factors that may influence male fertility there are relatively frequent pathologies that are characteristically highlighted during the first andrological screening visit of the individual. In particular, cryptorchidism and varicocele are main factors traditionally associated with testicular damage and fertility impairment, but also congenital abnormalities such as hypospadias should not be overlooked. It should also be remarked that these factors almost invariably overlap with other risk factors (such as smoking, alcohol consumption and obesity/physical activity) also known to influence andrological health of the individual. A critical aspect in the epidemiology of male factor infertility is that most of these aspects are often overlooked until the first andrological visit for infertility, which in developed countries may happen at a rather adult age (Pallotti et al., 2022). Adolescence and transition age is a critical window for testicular development and for maturation of the genitourinary tract (Abreu and Kaiser, 2016; Cargnelutti et al., 2022), and despite rising awareness there is still a marked gender gap in prevention programs for reproductive health and sexuality among young men and women (Gianfrilli et al., 2019). A recent survey in Italy, has highlighted that among final-year male high school students deleterious habits were relatively common (smoking 32.6%, drinking - 80.6%, illegal drugs - 46.5%) as well as overweight/obesity (near 20%) as well as the presence of abnormalities in the andrological physical examination (testicular hypotrophy - 14.0%, varicocele - 27.1%, phimosis - 7.1%) (Gianfrilli et al., 2019).

### 3.1. *Cryptorchidism*

Congenital cryptorchidism, that is the presence of one or both undescended testis at birth, is a relatively common congenital abnormality in males and its prevalence has been reported to range from 1% to 9% (Holmboe et al., 2024). In most cases, cryptorchid testes descend in the scrotum spontaneously within the first three months of life (around 80%), likely due the effects of gonadotropins, INSL-3 and testosterone during the so-called mini puberty. Exogenous hormone treatments may force the correct testes placement, but if this does not happen within the first months (ideally within 6 months), a surgical repositioning (orchidopexy) is warranted in order to prevent testicular damage in terms of spermatogenesis. A recent report highlighted how cryptorchidism prevalence varies among different geographical areas and a significant modification in its temporal trends (Holmboe et al., 2024). In particular, the authors reported that studies of cryptorchidism prevalence in the European geographical area detected a wide range of incidence (1.1% in Estonia, 2.4% in Denmark, 3.4% in Italy, 4.7% in Lithuania, 9.0% in Finland) while a prevalence of 2.1% was reported in the United States (Holmboe et al., 2024). The evaluation of the temporal trends in cryptorchidism is challenging due to different definitions and to not fully reliable registrations, but some countries registered a rise in prevalence such as in Denmark where historical data showed an increase from 1.8% in 1959–1961 to 8.5% in 1997–2001 (Boisen et al., 2004) or in France where the rate of orchiopexy increased more than 30% between 2004 and 2014 (Le Moal et al., 2021). On the other hand, in Italy a modest decline was registered (4.3% in 1978–1987 vs. 2.7% in 1988–1997) (Ghirri et al., 2002). Factors contributing to these geographical and temporal variations are unknown but are likely conducible to a combination of environmental exposures, lifestyle factors, and genetic/ethnic predispositions.

### 3.2. *Hypospadias*

Among other male non-genetic congenital abnormalities, hypospadias, the ectopic placement of the urethral opening along the ventral side of the penis, has been reported to have the highest prevalence rate. A population-based register study in Sweden reporting data collected from 1973 to 2009 described a prevalence of 1/125 male newborns (Nordenvall et al., 2014), while French registers including roughly a decade later (1981–2020) report a slightly lower prevalence (around 18 per 10,000 births) (Monier et al., 2024). Hypospadias etiology is rather complex, arising from different conditions which have the common factor of causing reduced levels of fetal androgens and, thus, low levels of dihydrotestosterone (DHT) that are unable to fully differentiate male external genitalia. Also depending on the specific etiology, hypospadias may correspond to the presence of hypogonadism and infertility in adulthood and a recent study also highlighted the possibility of an association of low-androgen comorbidities in adolescence and adulthood (delayed puberty, metabolic and cardiovascular diseases) (Phillips et al., 2022), which in turn may also further disrupt male fertility. Unsurprisingly, a population register study indicates clearly that men with hypospadias more often refer to ART centers to conceive a child (Skarin Nordenvall et al., 2020).

### 3.3. Varicocele

Varicocele is a common finding in an andrological screening for infertility and it presents as a unilateral (left in 78–93%, right in 1–7% of cases) or bilateral (2–20%) abnormal enlargement and tortuousness of the spermatic vein. In men of fertile age, its incidence is reported to range between 4.4 and 22.6%; in men undergoing fertility evaluation it can be detected in 21–41% of patients examined for primary fertility, and up to 75–81% of patients with secondary infertility (Pallotti et al., 2018). The correlation between impaired semen parameters, infertility and varicocele is well known and to this, several aspects may contribute including hypoxia, increased scrotal temperature, adrenal metabolite reflux, autoimmunity, increased oxidative stress and inflammation (Lund and Nielsen, 1996; Mostafa et al., 2009; Will et al., 2011; Finelli et al., 2021).

### 3.4. Lifestyle behaviour

Lifestyle and potentially harmful behaviors are an often underestimated cause of male infertility and it is good clinical practice for reproductive health specialists to carefully address this aspect (Ferlin et al., 2022). Obesity, heavy alcohol consumption, cigarette smoking, and other unhealthy habits are now recognized as modifiable risk factors for infertility. Cigarette smoking is indeed a global health problem, as the harmful effects of smoke on general and reproductive health are well known, with associations with impaired semen parameters widely documented in the scientific literature (Sharma et al., 2016). Furthermore, sperm protamine PRM1/2 ratios, protamination process during spermatogenesis, oxidative markers and sperm DNA integrity have also been documented to be negatively affected (Taha et al., 2012; Hamad et al., 2014 Szumilas et al., 2020). These same parameters appear to be significantly affected in heavy alcohol drinkers: a recent paper shows how negative effects on semen parameters and quality are more pronounced in heavy drinkers compared to smokers (Amor et al., 2022). It is likely that the association of the two habits in the same individual could have synergistic negative effects. Unhealthy diet and obesity also appear to be linked with impaired male reproductive outcomes. It should also be noted that the increase in reported fertility issues in the last decades has coincided with the increasing prevalence of obesity in men, and the associations of overweight/obesity and semen parameters impairment (Rotimi and Singh, 2024) further strengthen this link. Obese men often show endocrine alterations (see paragraph "Hypogonadism"), systemic inflammation and oxidative stress that are known to impair human fertility also in association with measurable biochemical and epigenetic biomarkers (George et al., 2023). The importance of these factors relies on the fact that they are clearly modifiable risk factors. On the other hand, the prevalence of most of them (obesity in particular) is known to be increasing. It is thus possible that, if these trends remain stable in the near future, the weight of lifestyle factors in reproductive health could dramatically increase.

### 3.5. Hypogonadism

Male hypogonadism is classified as both hypogonadotropic and hypergonadotropic, classically corresponding to the presence of a hypothalamic/pituitary or testicular disease, respectively. Moreover, causes of male hypogonadism are both congenital and acquired and these causes can broadly be considered as "organic" as they correspond to the presence of structural or genetic pathology of the hypothalamic-pituitary-gonadal axis. On the other hand, in recent years an increasing amount of evidence has highlighted the presence of a "functional" hypogonadotropic hypogonadism (FHH). This entity has been defined as a functional impairment of the hypothalamic-pituitary gonadal axis with low testosterone concentration together with inappropriately low (low or low-normal) levels of circulating FSH and LH, frequently associated with infertility (Spaziani et al., 2023). Once other "organic" causes of hypogonadism are excluded, a diagnosis of functional hypogonadotropic hypogonadism can be made. These rather heterogeneous forms of hypogonadism can be found in subjects with chronic diseases and or undergoing medical treatments but can also be detected in subjects with metabolic diseases and experiencing significant lifestyle changes (Cangiano et al., 2024). A precise etiology or pathogenetic mechanism is currently unclear. There is evidence that obese, dysmetabolic subjects may be prone to FHH due to testosterone aromatization in adipose tissue, and subsequent a relative excess of estrogens with reduced gonadotropin secretion through negative feedback, systemic inflammation and/or insulin/leptin resistance. Furthermore, a genetic predisposition is also possible, but most subjects with FHH may present with different pathological mechanism, especially in case of medical treatments (opioids, corticosteroids, dopamine-agonists, etc.) or overtraining with low fat mass and consistently negative energy balance (Spaziani et al., 2023).

### 3.6. Idiopathic infertility

In clinical practice, there are a remarkable number of couples in which non clear cause for infertility can be found. This condition is defined as idiopathic infertility and although the exact prevalence is hard to define, a relevant number of males from infertile couples present with sperm quality impairment classified as idiopathic or unexplained. A major impairment to an exact evaluation of the phenomena is bound to the fact that a thorough evaluation of the male partner is far too frequently overlooked (Cannarella et al., 2020). It has also been hypothesized that despite normal gonadotropin levels idiopathic infertile men could be affected by a form of functional hypogonadism Spaggiari et al. (2024). It should be noted that these undiagnosed conditions may be related to unhealthy habits including excessive food, alcohol and cigarette use as described in the previous paragraphs. Once systemic and andrological pathologies have been excluded, the putative etiology of this condition lies in sperm DNA and chromatin compaction alterations, as well as in still unknown genetic and epigenetic conditions (Heidary et al., 2020; Gunes and Esteves, 2021; Tang et al., 2022). It is still not possible to routinely include these molecular aspects in the work up of the infertile male, but nonetheless they represent possible future development for clinical practice. According to available evidence, it is possible to treat selected cases with antioxidants and nutraceuticals to contrast the presence of sperm DNA damage (Ferlin et al., 2022). Also eating habits and specifically high-fat diet could be related to sperm epigenetic alterations that can also be inherited by the offspring (Tomar et al., 2024). In general, it must be stressed that management of idiopathic infertility should start from correction of unhealthy lifestyles with possible impact on fertility. After this first step, it is possible to hypothesize further genetic investigations. More complex could be the treatment of genetic somatic alterations, but their knowledge could lead to more specific and tailored approaches to the infertile couple management.

## 4. Decline of male fertility - what has happened to seminal quality in the new millennium?

A relevant hypothesized trend of decline of male fertility has been debated in the last years. This aspect is strictly intertwined with the evolution of the incidence of male fertility disorders over the last decades as this has relevant repercussions for both individual health and broader socio-economic and demographic issues.

### 4.1. The history of the fertility alert

From an historical perspective, a '*fertility alert*' about declining sperm quality has been issued since the 1990s when Carlsen et al. raised public concern about the possibility of a decline in male fertility (Carlsen et al., 1992). This meta-analysis, evaluating 61 articles on semen characteristics from a total of 14,947 samples, found that the mean sperm count decreased by 50% between 1938 and 1991, indicating a general decline in semen quality (Carlsen et al., 1992). Despite this result was comparable with previous studies (Nelson and Bunge, 1974; MacLeod and Gold, 1951), Carlsen et al.'s meta-analysis sparked an open scientific debate which was followed up by more publications evaluating temporal trends in semen parameters in men with unknown or known fertility status, with conflicting results (Carlsen et al., 1992). The main criticisms concerned the criteria used in the selection of study participants, the methodology used for the semen analysis, the WHO reference values that changed over time and the statistical methods. In particular, Bahadur et al. (1996) questioned the reliability of the analysis, pointing to the importance of variability factors like time and country of origin, while Swan's group supported Carlsen et al. findings (Carlsen et al., 1992; Bahadur et al., 1996; Swan et al., 1997 Ravanos et al., 2018). Despite massive efforts, the published evidence remains contentious, with many confirmatory articles (Auger et al., 1995; Rolland et al., 2013) and many that report contradictory evidence of stability of semen parameters over the years (Bujan et al., 1996; Rasmussen et al., 1997; Axelsson et al., 2011; Jørgensen et al., 2012) or limit the decline to specific subgroups of patients (Gandini et al., 2000; Cargnelutti et al., 2023). However, it is difficult to prove global variations in sperm parameters because trends can vary from country to country and it is difficult to systematically compare different reports. (Sengupta et al., 2017). Many of these studies assume that the decline in semen quality over a given period and in given countries may be the same in all parts of the world. This is an unusual epidemiological concept; it is more logical to assume that the data obtained are only informative about the populations from which the data were collected, and if a decline is found, it is likely that it did not occur everywhere at the same time (Joffe, 2010). Moreover, most studies are heterogeneous and poorly matched; therefore, they are not objectively representative of the general population (Ravanos et al., 2018).

### 4.2. Possible confounding factors on fertility decline

Geographical distribution - The first papers describing variations in sperm concentration in men from New York, Texas, Iowa and Philadelphia, with higher values in New York and lower values in Iowa, were by Smith and Steinberger (1977) and MacLeod and Wang (1979). However, Fisch and Goluboff (1996) were the first to consider the possible confounding role of geographical distribution in the time course of semen quality. A retrospective analysis of semen quality in 4710 fertile unselected men and sperm bank donors provided evidence of regional differences in France (Auger and Jouannet, 1997); while in 2001 Jorgensen et al. found significant differences in sperm concentration and motility between samples from 4 European cities (Copenaghen, Edinburgh, Turku and Paris) (Jørgensen et al., 2001). Other studies have found differences in semen parameters between Denmark, Norway, Estonia and Finland (Jørgensen et al., 2002) and between Sweden and Denmark (Richthoff et al., 2002). These differences have been found not only in Europe, but also in four regions of Japan (Iwamoto et al., 2013). Feferkorn et al. (2022) have recently confirmed these findings. The authors found significant differences in all parameters between different geographical regions when analyzing semen parameters from studies used to define reference intervals for the WHO manual (2010, 2021). In this study, the decrease in semen parameters in Africa and Asia compared to Europe was on average 20.5% and 18% for semen volume respectively. For sperm concentration, the decrease in Africa compared to Europe was 49%, while in Australia there was a 32% increase in sperm concentration. Therefore, it is likely that significant geographical variation, rather than a decline over time, accounts for most of the differences in semen quality reported in several studies.

### 4.3. Study design

In addition, differences in study design (multicentre, randomisation of subjects, retrospective, observational, etc.), subject selection, may explain the different results between studies. Some studies selected men who had donated semen to sperm banks or had undergone evaluation for male factor infertility. These were not random samples of the general population and introduced selection bias (Cocuzza and Esteves, 2014).

### 4.4. Time frame of the studies

The period taken into account by these studies is another aspect that needs to be assessed. The majority of studies have evaluated the trend of sperm parameters over long periods of time, usually 15 years or more (Cargnelutti et al., 2023). The meta-analysis by Sengupta, showed a 32.5% decline in European men over a 50 years from 1965 to 2015 (Sengupta et al., 2018), while recently Levine et al. (2023) reported a decline in sperm count in unselected men from South/Central America-Asia-Africa over 38 years from 1981 to 2019. Longer-term studies may be more representative of trends over time, as they reduce the impact of unexplained short-term fluctuations in the overall data. However, some methodological issues need to be addressed (Auger et al., 2022). The methodology and experience of the technicians, the use of internal and external quality control and the difficulties in standardization of semen analysis are limiting factors in studies looking at trends over time. WHO criteria for semen analysis were introduced in 1980 and then revised in 1987, 1992, 1999, 2010 and 2021 with differences in sperm morphology and motility evaluation. In addition, many technological advances, particularly in the components of light microscopes, have been made over the last few decades (Cargnelutti et al., 2023). Some studies have evaluated semen parameters over a shorter period (10 years) (Giwercman and Bonde, 1998; Almagor et al., 2003; Marimuthu et al., 2003; Sripada et al., 2007; Axelsson et al., 2011; Borges et al., 2015; Centola et al., 2016; Li et al., 2016; Cannarella et al., 2021; Cargnelutti et al., 2023). This reduces potential biases. In particular Cargnelutti et al. conducted a monocentric study on 3329 subjects (1655 subjects with idiopathic infertility and 1674 with no confirmed andrological diseases) to evaluate total sperm number over the last ten 10 years. The assessment was carried out from 2010 to 2019. This period corresponds to the introduction and use of the World Health Organization, 2010 Manual. The semen analysis was carried out by two seminologists with the same level of training and using the same equipment; a quality control was carried out in accordance with World Health Organization, 2010. In this study, the association between smoking habit, age, BMI, seasonality, job, area of origin and birthplace and the total number of spermatozoa was evaluated in order to identify factors that may be detrimental. Many studies have collected data without controlling for confounding factors that may be relevant when analyzing a secular or geographical trend. The authors found a substantial stability of total sperm number during the last decade in healthy subjects and a significant negative effect of smoking habit and BMI on total sperm number in infertile men, suggesting that they are more "vulnerable" subjects to several negative factors, many of which are still unknown. Given this, Auger et al. (2022) suggested that the literature does not support the conclusion that human sperm quality is declining globally or in the western world. However, a trend can be observed in some specific areas. Environmental influences and different genetic backgrounds may also contribute to the differences in semen quality observed between countries and regions. In 2017, Erenpreiss et al. investigated semen quality in young men from the neighboring Baltic countries: Estonia, Latvia and Lithuania, including a subanalysis of data for two ethnicities in Estonia (Estonians and Russians) (Erenpreiss et al., 2017). This study found no differences in semen quality between men from the Baltic countries. However, ethnic Estonians had a higher total sperm count than Russians living in the same environment in Estonia did. Therefore, ethnic influences (genetic background) as well as environmental and lifestyle influences could be responsible for differences in semen quality. In fact, a limitation of studies evaluating semen quality over time is the lack of data on age, seasonality, drug use, diet, environment, and lifestyle, especially smoking, alcohol consumption and BMI, which could have an impact on semen parameters. In recent years, there has been growing concern about endocrine disrupting chemicals (EDCs) in the environment, in food and in consumer products, which can interfere with the biosynthesis and metabolism of hormones and alter the reproductive system. In fetal and neonatal life, exposure can also occur through the placenta and breastfeeding. Consequently, EDCs may affect human pre- and post-natal development. The biological effects of EDCs may vary considerably. They interfere with the hormonal binding to the androgen receptor (AR) or the estrogen receptor (ER) influencing cell apoptosis, proliferation, differentiation, carcinogenesis and inflammation (Cargnelutti et al., 2021). Animal studies have shown that some phthalates cause histological changes in the fetal testis. These changes include loss of Sertoli cells, disruption of the seminiferous tubules and formation of multinucleated germ cells. They may also induce Leydig cell dysfunction that leads to the inhibition of steroidogenic enzymes. For adults, the data on exposure to non-persistent EDCs appear consistent with spermatogenetic and hormonal disturbances, but the association is limited. The data are conflicting, with some papers suggesting a negative correlation with at least one of the semen parameters, or with no parameter at all (Pallotti et al., 2020). Unhealthy lifestyles such as smoking are also frequently associated with altered spermatogenesis. Data from the literature suggest that smokers have lower semen parameters than non-smokers, (Practice Committee of the American Society for Reproductive Medicine Fertil Steril. 2018). These negative effects have been confirmed by a meta-analysis with the confirmation of more marked effects on heavy smokers (Sharma et al., 2016). The combustion of tobacco produces about 4000 compounds, many of which have potentially toxic, mutagenic and carcinogenic effects (nicotine, cotinine, carbon monoxide, cadmium, polycyclic aromatic hydrocarbons etc.). Moreover, these substances may also damage male reproductive cells with a negative impact on reproductive processes (Pacifici et al., 1993; Pacifici et al., 1995; Gandini et al., 1997; Calogero et al., 2009). Cigarette combustion and smoke increase reactive oxygen species (ROS) in seminal plasma and cause oxidative damage to sperm cell membranes, ultimately affecting their structure and function (Barbagallo et al., 2024). In addition, these substances can interact both directly and indirectly with nucleic acids, causing DNA and protein adducts, inducing mutations and chromosomal abnormalities and epigenetic changes, even with possible long-term heritable effects on the offspring (Esakky and Moley, 2016). Likewise, cigarette smoking is demonstrated to have deleterious effects also on sperm DNA fragmentation (Amor et al., 2022). Therefore, known exogenous risk factors such as lifestyle, obesity, type II diabetes and sexually transmitted diseases should not be neglected in couples with fertility problems and infertile men. Regarding obesity, several meta-analyses have reported conflicting results: some meta-analyses have found a negative association between BMI and sperm quality, while others have failed to find this association (MacDonald et al., 2010; Sermondade et al., 2013; Campbell et al., 2015; Guo et al., 2017; Santi et al., 2024). Obesity is associated with a state of chronic inflammation and may indirectly contribute to the development of hypogonadism through increased production of inflammatory cytokines such as TNFalpha and IL-1beta by adipocytes, which may directly impair the ability of Leydig cells to produce testosterone (Cannarella et al., 2024). Obesity therefore, can induce oxidative stress in the testes and can cause oxidative damage to biomolecules, including lipids, proteins and DNA. Leisegang et al. (2021) reported an impact of obesity on sperm functional parameters; men with a BMI greater than 25 kg/m2 had a high sperm DNA fragmentation.

### 4.5. *Sperm epigenetic factors and future prospective*

In recent years, omics technologies have shown that the sperm is not a silent cell. We now know that histone modification, RNA and DNA methylation are all part of the sperm epigenetic machinery. Literature studies indicate that the reproductive risk is not so much related to the decrease in the cytological parameters (sperm number, motility and morphology), but to the molecular aspect of this cell, which can be modified by different conditions. Male gametes contain several factors that may be essential for fertilization and embryo development. These include sperm proteins, sperm organelles, sperm mRNA and sperm ncRNA (Conflitti et al., 2023). The miRNAs present in the sperm (sperm-borne miRNAs) appear to be involved in the control of several processes in reproductive biology, such as spermatogenesis, egg fertilization and embryo development (Salas-Huetos et al., 2020). Finocchi et al. found 4 miRNAs differentially expressed in seminal plasma of patients with obstructive and non-obstructive azoospermia and Klinefelter Syndrome in comparison to normozoospermic controls (Finocchi et al., 2020). In humans, miRNA-34c has been identified as the most abundant microRNA in mature spermatozoa (Alves et al., 2020). miRNA-34c regulates critical processes such as spermatogenesis, fertilization, and early embryonic development (Shi et al., 2020). Its expression is notably reduced in oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenoteratozoospermia, azoospermia (both obstructive and non-obstructive), Klinefelter syndrome, and idiopathic male infertility (Dorostghoal et al., 2022; Abu-Halima et al., 2014; Finocchi et al., 2020; Momeni et al., 2020).

Growing evidence suggests that miRNA-34c is also involved in the early stages of embryonic development through blocking BCL-2 (Liu et al., 2012). In humans, miR-34c-5p levels were found to be significantly correlated with a better embryo quality at day 3, higher implantation, pregnancy, birth rates (Cui et al., 2015) and embryo kinetics (Shi et al., 2020). The expression of miRNA-34c is frequently associated with that of miRNA-449b, likely due to their similar sequences, targets, and functions (Song et al., 2014). Similar to miRNA-34c, a downregulation of miRNA-449b was observed in testicular biopsies of infertile individuals (Abu-Halima et al., 2014). In bovine models, miRNA-449b-5p in spermatozoa contributes to cleavage kinetics, blastocyst formation, epigenetic reprogramming, and blastomere apoptosis (Wang et al., 2017). It has also been demonstrated that in murine models the ablation of miR-449a/b/c leads to overexpression of miR-34b/c, compensating for miR-449 deficiency (Yuan et al., 2019) suggesting a potential synergistic interaction between the two miRNAs. In human spermatozoa, Conflitti et al. found that higher levels of miR-34c-5p than miR-449b-5p increased the likelihood of viable embryos 14-fold (Conflitti et al., 2023). Through the inhibition of messenger RNAs, both miRNAs inhibit specific cyclins and cyclin-dependent kinases that suppress E2F1 activity. This induces the cell to exit the cell cycle to promote differentiation or apoptosis, depending on the cell conditions (Lizé et al., 2010; Bao et al., 2012; He et al., 2007). For this reason, miR-34c/449 are involved in ciliogenesis as they repress proteins that promote the cell cycle, allowing progenitor cells to differentiate into ciliated phenotypes (Yuan et al., 2019). Inactivation of miR-34b/c and miR-449 disrupts ciliogenesis, leading to erectile dysfunction, impaired spermatogenesis and male infertility (Yuan et al., 2019). In efferent ducts, defective ciliogenesis leads to abnormal fluid reabsorption which causes sperm aggregation and agglutination in the epididymis. Under these conditions, the number of spermatozoa reaching the epididymis is significantly reduced, leading to infertility conditions such as oligoasthenoteratozoospermia or azoospermia. In addition, dysregulated miRNA-34/449 levels directly affect spermatogenesis by altering the expression of target proteins, preventing germ cells from successfully entering meiosis and differentiating into mature spermatozoa (Pantos et al., 2021). This mechanism has been demonstrated in murine double knockout models for miRNA-34c and miRNA-449b, which exhibit low sperm counts, poor motility, and abnormal morphology, all contributing to male sterility. (Wu et al., 2014). Therefore, sperm DNA fragmentation and epigenetic changes (miRNAs) appear to play a role in key reproductive processes, such as embryo development. It is important to note that lifestyle, stress, aging and the environment can influence the spermatogenic process and alter the expression of miRNAs in spermatozoa and seminal plasma. This may directly alter the regulation of gene expression during early embryogenesis, but may also affect gene regulation in later stages of the offspring. The health and quality of the sperm provided at the time of fertilization play an important role (Soubry, 2015). This knowledge could result in a paradigm shift in the way we view reproductive success. The health effects of the environment might not be limited to expectant mothers, but be shared with male partners (Wu et al., 2015). These studies highlight the future sperm borne miRNAs and epigenetic changes may be useful tools as promising biomarkers for male infertility and ART outcomes. In fact, miRNAs represent promising therapeutic tools to restore impaired cellular functions. To date, several miRNA-based strategies have been developed. “Anti-miRNAs” are used to inhibit pathological miRNAs, while “miRNA mimics” restore the activity of miRNAs with suppressive function. These strategies open new perspectives for the development of targeted therapies based on microRNAs (Diener et al., 2022).

### 4.6. Translational perspectives and potential interventions

In the last years, literature on andrological diseases and worsening semen quality has fostered intense debate. However, despite a continuously growing body of data on fertility impairment and associated risk factors, researchers tend to remain anchored to a dichotomous and fragmented view of the etiological factors of male infertility. In this sense, the first milestone was set by World Health Organization, 2021 Manual (World Health Organization, 2021) that clearly stated that the fifth percentile of the proposed semen parameters distribution is not a cutoff between “fertile” and “infertile”: it should be considered, instead, a decision limit that needs to be enriched and further tailored by all the infertile couple’s clinical and biochemical data available, effectively empowering the clinical andrologist with the pivotal role of guiding the couple towards medical or assisted reproduction treatment. However, this active role requires a shift in the interpretative paradigm of the so-called “male factor infertility”. The dichotomic view must be replaced with one that goes beyond the opposition between “known” and “unexplained/idiopathic” causes and adopting an integrated and multidisciplinary model that starts from semen analysis and includes anatomical, endocrine, and lifestyle/environmental factors, also reaching emerging molecular markers, like sperm-borne microRNAs.

In summary, the wide problem of andrological diseases and their impact male reproductive health should be distributed in a model with at least three level of complexities (Fig. 1): the first level includes primarily the medical background of the subject, encompassing well-established andrological or endocrine conditions with a direct known impact on fertility (e.g., varicocele, hypogonadism, cryptorchidism, etc.); the second level should include all factors associated with a modulation of fertility, including lifestyle factors (eating and smoking habits), obesity, other toxic habits and environmental exposures, which interact with the pre-existing medical background of the male; the third level must then embrace the molecular aspects of the male, including genetic/epigenetic and other molecular factors. This last level is of extreme translational importance as it could represent the missing link between environmental exposure, spermatogenesis impairment and reduced/reducing reproductive potential, with a keen eye on altered expression of sperm-borne miRNAs (miR-34c, miR-449b) and sperm DNA fragmentation which could find direct applications in medically assisted reproduction as predictors of the ultimate success of the treatment. This integrated structure also offers a new interpretative key as what we currently consider idiopathic infertility could be in the future be reframed as a potentially molecular phenotype, currently undetectable by standard clinical methods but possibly identifiable through epigenetic markers. In this regard, sperm miRNAs may emerge as “second-generation biomarkers”, innovative tools with great potential both in the diagnostic workup and in the prognostic stratifications of couples in reproductive medicine. For example, as low miR-34c levels are associated with reduced embryo kinetics and worse success rates in ART procedures, after a thorough evaluation of the first two clinical levels, the integration of such markers in the workup of male infertility could have an impact on infertility risk even in apparently normozoospermic individuals. This could guide individualised pre-ART treatment strategies (nutraceuticals, antioxidants, gonadotropin treatments, varicocele repair, etc.) and, finally, improve sperm selection in ICSI protocols.

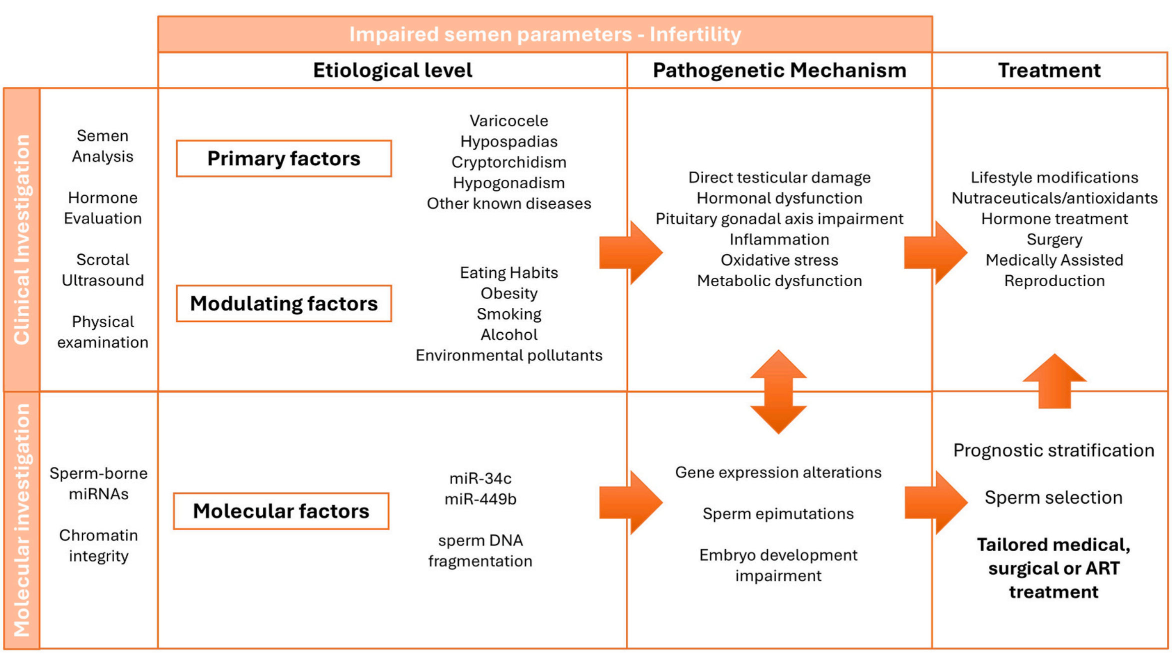


**Fig. 1.** Three level model of management of andrological diseases: diseases (primary factors), habits and environmental contributions (modulating factors), genetic factors and epigenetic responses (molecular factors).

## 5. Conclusions

Male Reproductive health can be considered an important and complex healthcare issue. It should be stressed that a number of conditions can significantly impact on reproductive outcomes, ranging from common andrological pathologies (varicocele, cryptorchidism) to unhealthy lifestyle habits (smoking, obesity, etc.). Furthermore, important confounding factors in reproductive habits could also arise from socioeconomic factors. Recent epidemiological data have suggested that overall human fertility has reduced, but available data may be biased from incomplete evaluation of possible metabolic factors that are indeed increasing in incidence in the last decades. It is thus imperative to maintain a rigorous approach to the study of male factor infertility in order to identify (and treat) andrological and metabolic confounders.

## CRediT authorship contribution statement

**Francesco Pallotti:** Writing – review & editing, Conceptualization. **Alessandra Buonacquisto:** Writing – review & editing, Data curation. **Gaia Cicolani:** Writing – review & editing, Data curation. **Anna Chiara Conflitti:** Writing – review & editing, Data curation. **Francesco Lombardo:** Writing – review & editing, Supervision. **Donatella Paoli:** Writing – review & editing, Conceptualization.

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## Conflicts of interest

None.

## References

Abreu, A.P., Kaiser, U.B., 2016. Pubertal development and regulation. Lancet Diabetes Endocrinol. 4 (3), 254–264. https://doi.org/10.1016/S2213-8587(15)00418-0.

Abu-Halima, M., Backes, C., Leidinger, P., Keller, A., Lubbad, A.M., Hammadeh, M., Meese, E., 2014. MicroRNA expression profiles in human testicular tissues of infertile men with different histopathologic patterns. Fertil. Steril. 101 (1), 78–86.e2. https://doi.org/10.1016/j.fertnstert.2013.09.009.

Almagor, M., Ivnitzki, I., Yaffe, H., Baras, M., 2003. Changes in semen quality in Jerusalem between 1990 and 2000: a cross-sectional and longitudinal study. Arch. Androl. 49 (2), 139–144. https://doi.org/10.1080/0148501039012.

Alves, M.B.R., Celeghini, E.C.C., Belleannée, C., 2020. From sperm motility to sperm-borne microRNA signatures: new approaches to predict Male fertility potential. Front. Cell Dev. Biol. 21 (8), 791. https://doi.org/10.3389/fcell.2020.00791.

Amor, H., Hammadeh, M.E., Mohd, I., Jankowski, P.M., 2022. Impact of heavy alcohol consumption and cigarette smoking on sperm DNA integrity. Andrologia 54 (7), e14434. https://doi.org/10.1111/and.14434.

Auger, J., Kunstmann, J.M., Czyglik, F., Jouannet, P., 1995. Decline in semen quality among fertile men in Paris during the past 20 years. N. Engl. J. Med. 332 (5), 281–285. https://doi.org/10.1056/NEJM199502023320501.

Auger, J., Jouannet, P., 1997. Evidence for regional differences of semen quality among fertile French men. Fédération Francaise des Centres d'Etude et de Conservation des Oeufs et du Sperme humains. Hum. Reprod. 12 (4), 740–745. https://doi.org/10.1093/humrep/12.4.740.

Auger, J., Eustache, F., Chevrier, C., Jégou, B., 2022. Spatiotemporal trends in human semen quality. Nat. Rev. Urol. 19 (10), 597–626. https://doi.org/10.1038/s41585-022-00626-w.

Axelsson, J., Rylander, L., Rignell-Hydbom, A., Giwercman, A., 2011. No secular trend over the last decade in sperm counts among Swedish men from the general population. Hum. Reprod. 26 (5), 1012–1016. https://doi.org/10.1093/humrep/der045.

Bahadur, G., Ling, K.L., Katz, M., 1996. Statistical modelling reveals demography and time are the main contributing factors in global sperm count changes between 1938 and 1996. Hum. Reprod. 11 (12), 2635–2639. https://doi.org/10.1093/oxfordjournals.humrep.a019184.

Bao, J., Li, D., Wang, L., Wu, J., Hu, Y., Wang, Z., Chen, Y., Cao, X., Jiang, C., Yan, W., Xu, C., 2012. MicroRNA-449 and microRNA-34b/c function redundantly in murine testes by targeting E2F transcription factor-retinoblastoma protein (E2F-pRb) pathway. J. Biol. Chem. 22 (287), 21686–21698. https://doi.org/10.1074/jbc.M111.328054, 26.

Barbagallo, F., Assenza, M.R., Torrisi, F., Buonacquisto, A., Pallotti, F., 2024. The smoky impact of nicotinic acetylcholine receptors on testicular function. J. Clin. Med. 13 (17), 5097. https://doi.org/10.3390/jcm13175097.

Boisen, K.A., Kaleva, M., Main, K.M., Virtanen, H.E., Haavisto, A.M., Schmidt, I.M., Chellakooty, M., Damgaard, I.N., Mau, C., Reunanen, M., Skakkebaek, N.E., Toppari, J., 2004. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet (London, England) 363 (9417), 1264–1269. https://doi.org/10.1016/S0140-6736(04)15998-9.

Borges Jr., E., Setti, A.S., Braga, D.P., Figueira, R.deC., Iaconelli Jr., A., 2015. Decline in semen quality among infertile men in Brazil during the past 10 years. Int. Braz. J. Urol. 41 (4), 757–763. https://doi.org/10.1590/S1677-5538.IBJU.2014.0186.

Bujan, L., Mansat, A., Pontonnier, F., Mieusset, R., 1996. Time series analysis of sperm concentration in fertile men in Toulouse, France between 1977 and 1992. BMJ 312 (7029), 471–472. https://doi.org/10.1136/bmj.312.7029.471.

Calogero, A., Polosa, R., Perdichizzi, A., Guarino, F., La Vignera, S., Scarfia, A., Fratantonio, E., Condorelli, R., Bonanno, O., Barone, N., Burrello, N., D'Agata, R., Vicari, E., 2009. Cigarette smoke extract immobilizes human spermatozoa and induces sperm apoptosis. Reprod. Biomed. Online 19 (4), 564–571. https://doi.org/10.1016/j.rbmo.2009.05.004.

Campbell, J.M., Lane, M., Owens, J.A., Bakos, H.W., 2015. Paternal obesity negatively affects male fertility and assisted reproduction outcomes: a systematic review and meta-analysis. Reprod. Biomed. Online 31 (5), 593–604. https://doi.org/10.1016/j.rbmo.2015.07.012.

Cangiano, B., Bonomi, M., Quinton, R., 2024. Editorial: functional acquired hypogonadotropic hypogonadism in males. Front. Endocrinol. (Lausanne) 15, 1364634. https://doi.org/10.3389/fendo.2024.1364634.

Cannarella, R., Condorelli, R.A., Mongioì, L.M., La Vignera, S., Calogero, A.E., 2020. Molecular biology of spermatogenesis: novel targets of apparently idiopathic Male infertility. Int. J. Mol. Sci. 3 (21), 1728. https://doi.org/10.3390/ijms21051728, 5.

Cannarella, R., Condorelli, R.A., Gusmano, C., Barone, N., Burrello, N., Aversa, A., Calogero, A.E., La Vignera, S., 2021. Temporal trend of conventional sperm parameters in a Sicilian population in the decade 2011-2020. J. Clin. Med. 10 (5), 993. https://doi.org/10.3390/jcm10050993.

Cannarella, R., Crafa, A., Curto, R., Condorelli, R.A., La Vignera, S., Calogero, A.E., 2024. Obesity and male fertility disorders. Mol. Aspect. Med. 97, 101273. https://doi.org/10.1016/j.mam.2024.101273. Advance online publication.

Cargnelutti, F., Di Nisio, A., Pallotti, F., Sabovic, I., Spaziani, M., Tarsitano, M.G., Paoli, D., Foresta, C., 2021. Effects of endocrine disruptors on fetal testis development, male puberty, and transition age. Endocrine 72 (2), 358–374. https://doi.org/10.1007/s12020-020-02436-9.

Cargnelutti, F., Di Nisio, A., Pallotti, F., Spaziani, M., Tarsitano, M.G., Paoli, D., Foresta, C., Talent Group, 2022. Risk factors on testicular function in adolescents. J. Endocrinol. Investig. 45 (9), 1625–1639. https://doi.org/10.1007/s40618-022-01769-8.

Cargnelutti, F., Pallotti, F., Carlini, T., Faja, F., Vestri, A.R., Fegatelli, D.A., Lenzi, A., Lombardo, F., Paoli, D., 2023. A decade of WHO 2010: total sperm number temporal trend and role of lifestyle factors. Asian J. Androl. 25 (5), 572–577. https://doi.org/10.4103/aja202297. Advance online publication.

Carlsen, E., Giwercman, A., Keiding, N., Skakkebaek, N.E., 1992. Evidence for decreasing quality of semen during past 50 years. BMJ 305 (6854), 609–613. https://doi.org/10.1136/bmj.305.6854.609.

Centola, G.M., Blanchard, A., Demick, J., Li, S., Eisenberg, M.L., 2016. Decline in sperm count and motility in young adult men from 2003 to 2013: observations from a U.S. sperm bank. Andrology 4 (2), 270–276. https://doi.org/10.1111/andr.12149.

Cocuzza, M., Esteves, S.C., 2014. Shedding light on the controversy surrounding the temporal decline in human sperm counts: a systematic review. Sci. World J., 365691 https://doi.org/10.1155/2014/365691.

Conflitti, A.C., Cicolani, G., Buonacquisto, A., Pallotti, F., Faja, F., Bianchini, S., Blaconà, G., Bruno, S.M., Linari, A., Lucarelli, M., Montanino, D., Muzii, L., Lenzi, A., Lombardo, F., Paoli, D., 2023. Sperm DNA fragmentation and sperm-borne miRNAs: molecular biomarkers of embryo development? Int. J. Mol. Sci. 24 (2), 1007. https://doi.org/10.3390/ijms24021007.

Cui, L., Fang, L., Shi, B., Qiu, S., Ye, Y., 2015. Spermatozoa micro ribonucleic acid-34c level is correlated with intracytoplasmic sperm injection outcomes. Fertil. Steril. 104 (2), 312–317.e1. https://doi.org/10.1016/j.fertnstert.2015.05.003.

Diener, C., Keller, A., Meese, E., 2022. Emerging concepts of miRNA therapeutics: from cells to clinic. Trends Genet. 38 (6), 613–626. https://doi.org/10.1016/j.tig.2022.02.006.

Dorostghoal, M., Galehdari, H., Hemadi, M.E., 2022. Sperm miR-34c-5p-5p transcript content and its Association with sperm parameters in unexplained infertile men. Reprod. Sci. 29, 84–90. https://doi.org/10.1007/s43032-021-00733-w.

Erenpreiss, J., Punab, M., Zilaitiene, B., Hlevicka, S., Zayakin, P., Matulevicius, V., Tomas Preiksa, R., Jørgensen, N., 2017. Semen quality of young men from the general population in Baltic countries. Hum. Reprod. 32 (6), 1334–1340. https://doi.org/10.1093/humrep/dex062.

Esakky, P., Moley, K.H., 2016. Paternal smoking and germ cell death: a mechanistic link to the effects of cigarette smoke on spermatogenesis and possible long-term sequelae in offspring. Mol. Cell. Endocrinol. 435, 85–93. https://doi.org/10.1016/j.mce.2016.07.015.

Feferkorn, I., Azani, L., Kadour-Peero, E., Hizkiyahu, R., Shrem, G., Salmon-Divon, M., Dahan, M.H., 2022. Geographic Variation in Semen Parameters from Data Used for the World Health Organization Semen Analysis Reference Ranges Fertil Steril, vol.118, pp. 475–482. https://doi.org/10.1016/j.fertnstert.2022.05.037, 3.

Ferlin, A., Calogero, A.E., Krausz, C., Lombardo, F., Paoli, D., Rago, R., Scarica, C., Simoni, M., Foresta, C., Rochira, V., Sbardella, E., Francavilla, S., Corona, G., 2022. Management of male factor infertility: position statement from the Italian Society of

Andrology and Sexual Medicine (SIAMS) : endorsing organization: italian society of embryology, reproduction, and research (SIERR). J. Endocrinol. Investig. 45 (5), 1085–1113. https://doi.org/10.1007/s40618-022-01741-6.

Ferlin, A., Garolla, A., Ghezzi, M., Selice, R., Palego, P., Caretta, N., Di Mambro, A., Valente, U., De Rocco Ponce, M., Dipresa, S., Sartori, L., Plebani, M., Foresta, C., 2021. Sperm count and hypogonadism as markers of general Male health. Eur. Urol. Focus 7 (1), 205–213. https://doi.org/10.1016/j.euf.2019.08.001.

Finelli, R., Pallotti, F., Cargnelutti, F., Faja, F., Carlini, T., Rizzo, F., Lenzi, A., Paoli, D., Lombardo, F., 2021. Sperm DNA damage and cytokines in varicocele: a case-control study. Andrologia 53 (5), e14023. https://doi.org/10.1111/and.14023.

Finocchi, F., Pelloni, M., Balercia, G., Pallotti, F., Radicioni, A.F., Lenzi, A., Lombardo, F., Paoli, D., 2020. Seminal plasma miRNAs in Klinefelter syndrome and in obstructive and non-obstructive azoospermia. Mol. Biol. Rep. 47 (6), 4373–4382. https://doi.org/10.1007/s11033-020-05552-x.

Fisch, H., Goluboff, E.T., 1996. Geographic variations in sperm counts: a potential cause of bias in studies of semen quality. Fertil. Steril. 65 (5), 1044–1046. https://doi.org/10.1016/s0015-0282(16)58284-3.

Gandini, L., Lombardo, F., Lenzi, A., Culasso, F., Pacifici, R., Zuccaro, P., Dondero, F., 1997. The in-vitro effects of nicotine and cotinine on sperm motility. Hum. Reprod. 12 (4), 727–733. https://doi.org/10.1093/humrep/12.4.727.

Gandini, L., Lombardo, F., Culasso, F., Dondero, F., Lenzi, A., 2000. Myth and reality of the decline in semen quality: an example of the relativity of data interpretation. J. Endocrinol. Investig. 23 (6), 402–411. https://doi.org/10.1007/BF03343745.

George, B.T., Jhancy, M., Dube, R., Kar, S.S., Annamma, L.M., 2023. The molecular basis of Male infertility in obesity: a literature review. Int. J. Mol. Sci. 25 (1), 179. https://doi.org/10.3390/ijms25010179.

Ghirri, P., Ciulli, C., Vuerich, M., Cuttano, A., Faraoni, M., Guerrini, L., Spinelli, C., Tognetti, S., Boldrini, A., 2002. Incidence at birth and natural history of cryptorchidism: a study of 10,730 consecutive male infants. J. Endocrinol. Investig. 25 (8), 709–715. https://doi.org/10.1007/BF03345105.

Gianfrilli, D., Ferlin, A., Isidori, A.M., Garolla, A., Maggi, M., Pivonello, R., Santi, D., Sansone, A., Balercia, G., Granata, A.R.M., Sinisi, A., Lanfranco, F., Pasqualetti, P., Foresta, C., Lenzi, A., Amico-Andrologo' Study Group, 2019. Risk behaviours and alcohol in adolescence are negatively associated with testicular volume: results from the amico-andrologo survey. Andrology 7 (6), 769–777. https://doi.org/10.1111/andr.12659.

Giwercman, A., Bonde, J.P., 1998. Declining male fertility and environmental factors. Endocrinol Metab. Clin. N. Am. 27 (4), 807–viii. https://doi.org/10.1016/s0889-8529(05)70042-6.

Guo, D., Wu, W., Tang, Q., Qiao, S., Chen, Y., Chen, M., Teng, M., Lu, C., Ding, H., Xia, Y., Hu, L., Chen, D., Sha, J., Wang, X., 2017. The impact of BMI on sperm parameters and the metabolite changes of seminal plasma concomitantly. Oncotarget 8 (30), 48619–48634. https://doi.org/10.18632/oncotarget.14950.

Gunes, S., Esteves, S.C., 2021. Role of genetics and epigenetics in male infertility. Andrologia 53 (1), e13586. https://doi.org/10.1111/and.13586.

Hamad, M.F., Shelko, N., Kartarius, S., Montenarh, M., Hammadeh, M.E., 2014. Impact of cigarette smoking on histone (H2B) to protamine ratio in human spermatozoa and its relation to sperm parameters. Andrology 2 (5), 666–677. https://doi.org/10.1111/j.2047-2927.2014.00245.x.

He, L., He, X., Lim, L.P., de Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., Ridzon, D., Jackson, A.L., Linsley, P.S., Chen, C., Lowe, S.W., Cleary, M. A., Hannon, G.J., 2007. A microRNA component of the p53 tumour suppressor network. Nature 28 (447), 1130–1134. https://doi.org/10.1038/nature05939, 7148.

Heidary, Z., Saliminejad, K., Zaki-Dizaji, M., Khorram Khorshid, H.R., 2020. Genetic aspects of idiopathic asthenozoospermia as a cause of male infertility. Hum Fertil (Camb) 23 (2), 83–92. https://doi.org/10.1080/14647273.2018.1504325.

Holmboe, S.A., Beck, A.L., Andersson, A.M., Main, K.M., Jørgensen, N., Skakkebæk, N.E., Priskorn, L., 2024. The epidemiology of cryptorchidism and potential risk factors, including endocrine disrupting chemicals. Front. Endocrinol.(Lausanne) 15, 1343887. https://doi.org/10.3389/fendo.2024.1343887.

ISTAT, 2021. http://dati.istat.it/.

Iwamoto, T., Nozawa, S., Yoshiike, M., Namiki, M., Koh, E., Kanaya, J., Okuyama, A., Matsumiya, K., Tsujimura, A., Komatsu, K., Tsukamoto, T., Itoh, N., Mieno, M.N., Vierula, M., Toppari, J., Skakkebæk, N.E., Jørgensen, N., 2013. Semen quality of fertile Japanese men: a cross-sectional population-based study of 792 men. BMJ Open (1), e002223. https://doi.org/10.1136/bmjopen-2012-002223.

Joffe, M., 2010. What has happened to human fertility? Hum. Reprod. 25 (2), 295–307. https://doi.org/10.1093/humrep/dep390.

Jørgensen, N., Andersen, A.G., Eustache, F., Irvine, D.S., Suominen, J., Petersen, J.H., Andersen, A.N., Auger, J., Cawood, E.H., Horte, A., Jensen, T.K., Jouannet, P., Keiding, N., Vierula, M., Toppari, J., Skakkebaek, N.E., 2001. Regional differences in semen quality in Europe. Hum. Reprod. 16 (5), 1012–1019. https://doi.org/10.1093/humrep/16.5.1012.

Jørgensen, N., Carlsen, E., Nermoen, I., Punab, M., Suominen, J., Andersen, A.G., Andersson, A.M., Haugen, T.B., Horte, A., Jensen, T.K., Magnus, Ø., Petersen, J.H., Vierula, M., Toppari, J., Skakkebaek, N.E., 2002. East-West gradient in semen quality in the nordic-baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum. Reprod. 17 (8), 2199–2208. https://doi.org/10.1093/humrep/17.8.2199.

Jørgensen, N., Joensen, U.N., Jensen, T.K., Jensen, M.B., Almstrup, K., Olesen, I.A., Juul, A., Andersson, A.M., Carlsen, E., Petersen, J.H., Toppari, J., Skakkebæk, N.E., 2012. Human semen quality in the new millennium: a prospective cross-sectional population-based study of 4867 men. BMJ Open 2 (4), e000990. https://doi.org/10.1136/bmjopen-2012-000990.

Le Moal, J., Goria, S., Guillet, A., Rigou, A., Chesneau, J., 2021. Time and spatial trends of operated cryptorchidism in France and environmental hypotheses: a nationwide study from 2002 to 2014. Hum. Reprod. (Oxford, England) 36 (5), 1383–1394. https://doi.org/10.1093/humrep/deaa378.

Leisegang, K., Sengupta, P., Agarwal, A., Henkel, R., 2021. Obesity and male infertility: mechanisms and management. Andrologia 53 (1), e13617. https://doi.org/10.1111/and.13617.

Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Jolles, M., Pinotti, R., Swan, S.H., 2023. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. Hum. Reprod. Update 29 (2), 157–176. https://doi.org/10.1093/humupd/dmac035.

Li, C.J., Tzeng, C.R., Chen, R.Y., Han, B.C., Yeh, C.Y., Chien, L.C., 2016. Decline in semen quality in men in northern Taiwan between 2001 and 2010. Chin. J. Physiol. 59 (6), 355–365. https://doi.org/10.4077/CJP.2016.BAF441.

Liu, W.M., Pang, R.T., Chiu, P.C., Wong, B.P., Lao, K., Lee, K.F., Yeung, W.S., 2012. Sperm-borne microRNA-34c is required for the first cleavage division in mouse. Proc. Natl. Acad. Sci. USA 10 (109), 490–494. https://doi.org/10.1073/pnas.1110368109, 2.

Lizé, M., Pilarski, S., Dobbelstein, M., 2010. E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis. Cell Death Differ. 17 (3), 452–458. https://doi.org/10.1038/cdd.2009.188.

Lund, L., Nielsen, K.T., 1996. Varicocele testis and testicular temperature. Br. J. Urol. 78 (1), 113–115. https://doi.org/10.1046/j.1464-410x.1996.05122.x.

MacDonald, A.A., Herbison, G.P., Showell, M., Farquhar, C.M., 2010. The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. Hum. Reprod. Update 16 (3), 293–311. https://doi.org/10.1093/humupd/dmp047.

MacLeod, J., Gold, R.Z., 1951. The male factor in fertility and infertility. III. An analysis of motile activity in the spermatozoa of 1000 fertile men and 1000 men in infertile marriage. Fertil. Steril. 2 (3), 187–204.

MacLeod, J., Wang, Y., 1979. Male fertility potential in terms of semen quality: a review of the past, a study of the present. Fertil. Steril. 31 (2), 103–116. https://doi.org/10.1016/s0015-0282(16)43808-2.

Marimuthu, P., Kapilashrami, M.C., Misro, M.M., Singh, G., 2003. Evaluation of trend in semen analysis for 11 years in subjects attending a fertility clinic in India. Asian J. Androl. 5 (3), 221–225.

Monier, I., Hachem, S., Goffinet, F., Martinez-Marin, A., Khoshnood, B., Lelong, N., 2024. Population-based surveillance of congenital anomalies over 40 years (1981-2020): results from the Paris registry of congenital malformations (remaPAR). J. Gynecol. Obstet. Hum. Reprod. 53 (6), 102780. https://doi.org/10.1016/j.jogoh.2024.102780.

Momeni, A., Najafipour, R., Hamta, A., Jahani, S., Moghbelinejad, S., 2020. Expression and methylation pattern of hsa-miR-34 family in sperm samples of infertile men. Reprod. Sci. 27 (1), 301–308. https://doi.org/10.1007/s43032-019-00025-4.

Mostafa, T., Anis, T., Imam, H., El-Nashar, A.R., Osman, I.A., 2009. Seminal reactive oxygen species-antioxidant relationship in fertile males with and without varicocele. Andrologia 41 (2), 125–129. https://doi.org/10.1111/j.1439-0272.2008.00900.x.

Nelson, C.M., Bunge, R.G., 1974. Semen analysis: evidence for changing parameters of male fertility potential. Fertil. Steril. 25 (6), 503–507. https://doi.org/10.1016/s0015-0282(16)40454-1.

Nordenvall, A.S., Frisén, L., Nordenström, A., Lichtenstein, P., Nordenskjöld, A., 2014. Population based nationwide study of hypospadias in Sweden, 1973 to 2009: incidence and risk factors. J. Urol. 191 (3), 783–789. https://doi.org/10.1016/j.juro.2013.09.058.

Pacifici, R., Altieri, I., Gandini, L., Lenzi, A., Pichini, S., Rosa, M., Zuccaro, P., Dondero, F., 1993. Nicotine, cotinine, and trans-3-hydroxycotinine levels in seminal plasma of smokers: effects on sperm parameters. Ther. Drug Monit. 15 (5), 358–363. https://doi.org/10.1097/00007691-199310000-00002.

Pacifici, R., Altieri, I., Gandini, L., Lenzi, A., Passa, A.R., Pichini, S., Rosa, M., Zuccaro, P., Dondero, F., 1995. Environmental tobacco smoke: nicotine and cotinine concentration in semen. Environ. Res. 68 (1), 69–72. https://doi.org/10.1006/enrs.1995.1009.

Pallotti, F., Paoli, D., Carlini, T., Vestri, A.R., Martino, G., Lenzi, A., Lombardo, F., 2018. Varicocele and semen quality: a retrospective case-control study of 4230 patients from a single centre. J. Endocrinol. Investig. 41 (2), 185–192. https://doi.org/10.1007/s40618-017-0713-z.

Pallotti, F., Pelloni, M., Gianfrilli, D., Lenzi, A., Lombardo, F., Paoli, D., 2020. Mechanisms of testicular disruption from exposure to bisphenol A and phtalates. J. Clin. Med. 9 (2), 471. https://doi.org/10.3390/jcm9020471.

Pallotti, F., Barbonetti, A., Rastrelli, G., Santi, D., Corona, G., Lombardo, F., 2022. The impact of male factors and their correct and early diagnosis in the infertile couple's pathway: 2021 perspectives. J. Endocrinol. Investig. 45 (10), 1807–1822. https://doi.org/10.1007/s40618-022-01778-7.

Pantos, K., Grigoriadis, S., Tomara, P., Louka, I., Maziotis, E., Pantou, A., Nitsos, N., Vaxevanoglou, T., Kokkali, G., Agarwal, A., Sfakianoudis, K., Simopoulou, M., 2021. Investigating the role of the microRNA-34/449 family in Male infertility: a critical analysis and review of the literature. Front. Endocrinol. (Lausanne) 1 (12), 709943. https://doi.org/10.3389/fendo.709943.

Petre, G.C., Francini-Pesenti, F., Di Nisio, A., De Toni, L., Grande, G., Mingardi, A., Cusmano, A., Spinella, P., Ferlin, A., Garolla, A., 2023. Observational cross-sectional study on mediterranean diet and sperm parameters. Nutrients 15 (23), 4989. https://doi.org/10.3390/nu15234989.

Phillips, L., Lundholm, C., Kvist, U., Almqvist, C., Nordenskjöld, A., Skarin Nordenvall, A., 2022. Increased androgen-related comorbidity in adolescents and adults born with hypospadias: a population-based study. Andrology 10 (7), 1376–1386. https://doi.org/10.1111/andr.13229.

Practice Committee of the American Society for Reproductive Medicine, 2018. Electronic address: asrm@asrm.org, & practice committee of the American society for reproductive medicine, 2018. Smoking and infertility: a committee opinion. Fertil. Steril. 110 (4), 611–618. https://doi.org/10.1016/j.fertnstert.2018.06.016.

Rasmussen, P.E., Erb, K., Westergaard, L.G., Laursen, S.B., 1997. No evidence for decreasing semen quality in four birth cohorts of 1,055 Danish men born between 1950 and 1970. Fertil. Steril. 68 (6), 1059–1064. https://doi.org/10.1016/s0015-0282(97)00377-4.

Ravanos, K., Petousis, S., Margioula-Siarkou, C., Papatheodorou, A., Panagiotidis, Y., Prapas, N., Prapas, Y., 2018. Declining sperm counts… or rather not? A mini review. Obstet. Gynecol. Surv. 73 (10), 595–605. https://doi.org/10.1097/OGX.0000000000000599.

Richthoff, J., Rylander, L., Hagmar, L., Malm, J., Giwercman, A., 2002. Higher sperm counts in Southern Sweden compared with Denmark. Hu Reprod 17 (9), 2468–2473. https://doi.org/10.1093/humrep/17.9.2468.

Rolland, M., Le Moal, J., Wagner, V., Royère, D., De Mouzon, J., 2013. Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. Hum. Reprod. 28 (2), 462–470. https://doi.org/10.1093/humrep/des415.

Rotimi, D.E., Singh, S.K., 2024. Implications of lifestyle factors on male reproductive health. JBRA Assist. Reprod. 28 (2), 320–330. https://doi.org/10.5935/1518-0557.20240007.

Salas-Huetos, A., Bulló, M., Salas-Salvadó, J., 2017. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. Hum. Reprod. Update 23 (4), 371–389. https://doi.org/10.1093/humupd/dmx006.

Salas-Huetos, A., James, E.R., Aston, K.I., Carrell, D.T., Jenkins, T.G., Yeste, M., 2020. The role of miRNAs in male human reproduction: a systematic review. Andrology 8 (1), 7–26. https://doi.org/10.1111/andr.12714.

Santi, D., Lotti, F., Sparano, C., Rastrelli, G., Isidori, A.M., Pivonello, R., Barbonetti, A., Salonia, A., Minhas, S., Krausz, C., Vignozzi, L., Maggi, M., Corona, G., 2024. Does an increase in adipose tissue 'weight' affect male fertility? A systematic review and meta-analysis based on semen analysis performed using the WHO 2010 criteria. Andrology 12 (1), 123–136. https://doi.org/10.1111/andr.13460.

Sengupta, P., Dutta, S., Krajewska-Kulak, E., 2017. The disappearing sperms: analysis of reports published between 1980 and 2015. Am. J. Mens Health 11 (4), 1279–1304. https://doi.org/10.1177/1557988316643383.

Sengupta, P., Borges Jr., E., Dutta, S., Krajewska-Kulak, E., 2018. Decline in sperm count in European men during the past 50 years. Hum. Exp. Toxicol. 37 (3), 247–255. https://doi.org/10.1177/0960327117703690.

Sermondade, N., Faure, C., Fezeu, L., Shayeb, A.G., Bonde, J.P., Jensen, T.K., Van Wely, M., Cao, J., Martini, A.C., Eskandar, M., Chavarro, J.E., Koloszar, S., Twigt, J. M., Ramlau-Hansen, C.H., Borges Jr., E., Lotti, F., Steegers-Theunissen, R.P., Zorn, B., Polotsky, A.J., La Vignera, S., et al., 2013. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. Hum. Reprod. Update 19 (3), 221–231. https://doi.org/10.1093/humupd/dms050.

Sharma, R., Harlev, A., Agarwal, A., Esteves, S.C., 2016. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 world health organization laboratory methods for the examination of human semen. Eur. Urol. 70 (4), 635–645. https://doi.org/10.1016/j.eururo.2016.04.010.

Shi, S., Shi, Q., Sun, Y., 2020. The effect of sperm miR-34c on human embryonic development kinetics and clinical outcomes. Life Sci. 1 (256), 117895. https://doi.org/10.1016/j.lfs.2020.117895.

Skarin Nordenvall, A., Chen, Q., Norrby, C., Lundholm, C., Frisén, L., Nordenström, A., Almqvist, C., Nordenskjöld, A., 2020. Fertility in adult men born with hypospadias: a nationwide register-based cohort study on birthrates, the use of assisted reproductive technologies and infertility. Andrology 8 (2), 372–380. https://doi.org/10.1111/andr.12723.

Smith, K.D., Steinberger, E., 1977. What is oligospermia? In: Troen, P., Nankin, H.R. (Eds.), The Testis in Normal and Infertile Men. Raven Press, New York, pp. 489–503.

Song, R., Walentek, P., Sponer, N., Klimke, A., Lee, J.S., Dixon, G., Harland, R., Wan, Y., Lishko, P., Lize, M., Kessel, M., He, L., 2014. miR-34/449 miRNAs are required for motile ciliogenesis by repressing cp110. Nature 5 (510), 115–120. https://doi.org/10.1038/nature13413, 7503.

Soubry, A., 2015. Epigenetic inheritance and evolution: a paternal perspective on dietary influences. Prog. Biophys. Mol. Biol. 118 (1–2), 79–85. https://doi.org/10.1016/j.pbiomolbio.2015.02.008.

Spaggiari, G., Costantino, F., Dalla Valentina, L., Romeo, M., Furini, C., Roli, L., De Santis, M.C., Canu, G., Trenti, T., Granata, A.R.M., Simoni, M., Santi, D., 2024. Are they functional hypogonadal men? Testosterone serum levels unravel male idiopathic infertility subgroups. Endocrine 84 (2), 757–767. https://doi.org/10.1007/s12020-024-03717-3.

Spaziani, M., Carlomagno, F., Tarantino, C., Angelini, F., Vincenzi, L., Gianfrilli, D., 2023. New perspectives in functional hypogonadotropic hypogonadism: beyond late onset hypogonadism. Front. Endocrinol. (Lausanne) 14, 1184530. https://doi.org/10.3389/fendo.2023.1184530.

Sripada, S., Fonseca, S., Lee, A., Harrild, K., Giannaris, D., Mathers, E., Bhattacharya, S., 2007. Trends in semen parameters in the northeast of Scotland. J. Androl. 28 (2), 313–319. https://doi.org/10.2164/jandrol.106.000729.

Swan, S.H., Elkin, E.P., Fenster, L., 1997. Have sperm densities declined? A reanalysis of global trend data. Environ. Health Perspect. 105 (11), 1228–1232. https://doi.org/10.1289/ehp.971051228.

Szumilas, K., Szumilas, P., Grzywacz, A., Wilk, A., 2020. The effects of E-Cigarette vapor components on the morphology and function of the Male and female reproductive systems: a systematic review. Int. J. Environ. Res. Publ. Health 17 (17), 6152. https://doi.org/10.3390/ijerph17176152.

Taha, E.A., Ez-Aldin, A.M., Sayed, S.K., Ghandour, N.M., Mostafa, T., 2012. Effect of smoking on sperm vitality, DNA integrity, seminal oxidative stress, zinc in fertile men. Urology 80 (4), 822–825. https://doi.org/10.1016/j.urology.2012.07.002.

Tang, D., Li, K., Geng, H.C., Lv, M., Gao, Y., Wang, G., Yu, H., Shao, Z., Shen, Q., Jiang, H., Zhang, X., He, X., Cao, Y., 2022. Identification of deleterious variants in patients with male infertility due to idiopathic non-obstructive azoospermia. Reprod. Biol. Endocrinol. 2 (20), 63. https://doi.org/10.1186/s12958-022-00950-1 doi: 10.1186/s12958-022-00936-z. Erratum in: Reprod Biol Endocrinol. 2022 May 20;20 (1):79.

Tomar, A., Gomez-Velazquez, M., Gerlini, R., Comas-Armangué, G., Makharadze, L., Kolbe, T., Boersma, A., Dahlhoff, M., Burgstaller, J.P., Lassi, M., Darr, J., Toppari, J., Virtanen, H., Kühnapfel, A., Scholz, M., Landgraf, K., Kiess, W., Vogel, M., Gailus-Durner, V., Fuchs, H., Marschall, S., Hrabě de Angelis, M., Kotaja, N., Körner, A., Teperino, R., 2024. Epigenetic inheritance of diet-induced and sperm-borne mitochondrial RNAs. Nature 630 (8017), 720–727. https://doi.org/10.1038/s41586-024-07472-3. Jun.

Venigalla, G., Ila, V., Dornbush, J., Bernstein, A., Loloi, J., Pozzi, E., Miller, D., Ramasamy, R., 2023. Male obesity: associated effects on fertility and the outcomes of offspring. Andrology. https://doi.org/10.1111/andr.13552, 10.1111/andr.13552. Advance online publication.

Wang, M., Gao, Y., Qu, P., Qing, S., Qiao, F., Zhang, Y., Mager, J., Wang, Y., 2017. Sperm-borne miR-449b influences cleavage, epigenetic reprogramming and apoptosis of SCNT embryos in bovine. Sci. Rep. 17 (7), 13403. https://doi.org/10.1038/s41598-017-13899-8, 1.

Will, M.A., Swain, J., Fode, M., Sonksen, J., Christman, G.M., Ohl, D., 2011. The great debate: Varicocele treatment and impact on fertility. Fertil. Steril. 95 (3), 841–852. https://doi.org/10.1016/j.fertnstert.2011.01.002.

World Health Organization, 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen, fifth ed. World Health Organization, Geneva.

World Health Organization, 2021. WHO Laboratory Manual for the Examination and Processing of Human Semen, sixth ed. World Health Organization, Geneva.

Wu, J., Bao, J., Kim, M., Yuan, S., Tang, C., Zheng, H., Mastick, G.S., Xu, C., Yan, W., 2014. Two miRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis. Proc. Natl. Acad. Sci. USA 15 (111), E2851–E2857. https://doi.org/10.1073/pnas.1407777111, 28.

Wu, H., Hauser, R., Krawetz, S.A., Pilsner, J.R., 2015. Environmental susceptibility of the sperm epigenome during windows of Male germ cell development. Curr. Environ. Health Rep. 2 (4), 356–366. https://doi.org/10.1007/s40572-015-0067-7.

Yuan, S., Liu, Y., Peng, H., Tang, C., Hennig, G.W., Wang, Z., Wang, L., Yu, T., Klukovich, R., Zhang, Y., Zheng, H., Xu, C., Wu, J., Hess, R.A., Yan, W., 2019. Motile cilia of the male reproductive system require miR-34/miR-449 for development and function to generate luminal turbulence. Proc. Natl. Acad. Sci. USA 26 (116), 3584–3593. https://doi.org/10.1073/pnas.1817018116, 9.