# Male Infertility and the Future of In Vitro Fertilization

Brent M. Hanson, MD, Daniel J. Kaser, MD,
Jason M. Franasiak, MD, HCLD/ALD*

**KEYWORDS**

• Assisted reproductive technology • Epigenetics • Genetics • Intracytoplasmic sperm injection
• In vitro fertilization • Male factor infertility

**KEY POINTS**

- A diagnosis of male factor infertility is associated with epigenetic changes, which may affect reproductive outcomes and could potentially impact the health of future generations.
- Genetic mutations likely play a role in male fertility, but individual polymorphisms only contribute to a small percentage of all male infertility cases.
- Cryopreservation affects semen analysis parameters and sperm DNA integrity, but the clinical superiority of fresh sperm over frozen sperm has not been firmly established.
- Obesity among men of reproductive age is becoming increasingly prevalent and seems to have a detrimental impact on fertility potential.
- The role of paternal age on sperm quality and fertility outcomes is controversial and difficult to assess due to confounders arising from the female partner.

## INTRODUCTION

The male partner's role in infertility has been the subject of increased investigation over the last several years.[1] Although the female partner has historically been the primary focus of an infertility evaluation, it is now clear that early recognition and treatment of male factor infertility substantially improves a couple's chances of success with fertility treatment. Approximately 20% of couple infertility can be attributed solely to the male, and a male factor is believed to contribute at least partially to difficulties with achieving pregnancy in as many as 50% of infertile couples.[2]

Since the birth of the first child conceived through in vitro fertilization (IVF) in 1978, physicians and researchers have made significant advancements within the field of infertility.[3] In modern society, the use of assisted reproductive technology (ART) is now commonplace. Between 1987 and 2015, it was reported that 1 million babies were born through the use of IVF or ART in the United States, and the percentage of births arising from ART has been rapidly increasing.[4] In 2015, 1.7% of all infants born in the United States and 4.5% of births in the state of Massachusetts resulted from ART.[5] As of 2019, the total number of births achieved through ART likely exceeds 8 million globally.[6]

The general population's overall acceptance of IVF as a treatment modality likely stems from improvements which have been observed in IVF outcomes. IVF protocols have undergone a tremendous evolution over the years, resulting in successful family building for infertile couples. Optimization of both laboratory techniques and clinical practice has led to dramatic improvements

IVI-RMA New Jersey, Sidney Kimmel Medical College at Thomas Jefferson University, 140 Allen Road, Basking Ridge, NJ 07920, USA
* Corresponding author.
*E-mail address:* jfranasiak@ivirma.com

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in live birth rates after IVF. Based on preliminary data from the 2017 National Summary Report from the Society for Assisted Reproductive Technology, in women less than 35 years old using autologous oocytes, 46.8% of all initiated IVF cycles in the United States resulted in live births.[7] This is a significant progress considering the IVF pregnancy rate of 6% originally reported by Edwards and colleagues in 1980.[8] From the male perspective, technological advancements, such as an intracytoplasmic sperm injection (ICSI), first introduced in 1992, have made it possible for couples with severe male factor infertility or failed fertilization in previous IVF cycles to achieve pregnancy.[9]

A recent trend within the field has been to minimize multiple gestations while increasing delivery rates and improving obstetric outcomes for singleton pregnancies.[10] Attempts to achieve these goals have primarily focused on interventions related to the female partner or the IVF laboratory. Single embryo transfer at the blastocyst stage, the use of preimplantation genetic testing, and the concept of achieving embryo and endometrial synchrony through freeze-all cycles have been described as potential techniques to improve patient outcomes and have been incorporated into many clinical practices.[11–14] To further improve IVF outcomes going forward, a focus on the male contribution to ART is crucial. This article will specifically highlight several topics related to male reproductive biology and will discuss how the genetic, epigenetic, and clinical aspects of male factor infertility are intrinsically linked to current IVF practice and the future success of IVF.

## THE RELATIONSHIP BETWEEN EPIGENETICS, TRANSGENERATIONAL EPIGENETIC INHERITANCE, AND IN VITRO FERTILIZATION

The term epigenetics was coined in the 1940s to describe interactions between genes and the environment that could not be fully explained through classic genetics.[15] Today, the concept of epigenetics primarily refers to 2 major types of modifications that occur in chromatin: DNA methylation and posttranslational histone modifications.[16] Epigenetic modifications are responsible for controlling numerous processes within humans and serve an important regulatory role within the male reproductive system.[17] It is thought that the epigenetic remodeling that occurs during late spermiogenesis, primarily the sequential replacement of histones by protamines, protects sperm DNA from oxidative stress arising from exposure to the female reproductive tract.[18]

As understanding of the sperm epigenome has increased, there has been a growing body of evidence supporting a link between abnormal epigenetic sperm methylation patterns and male factor infertility (Fig. 1).[19] Through the use of arrays or targeted sequencing after bisulfate conversion, various loci have been evaluated for associations

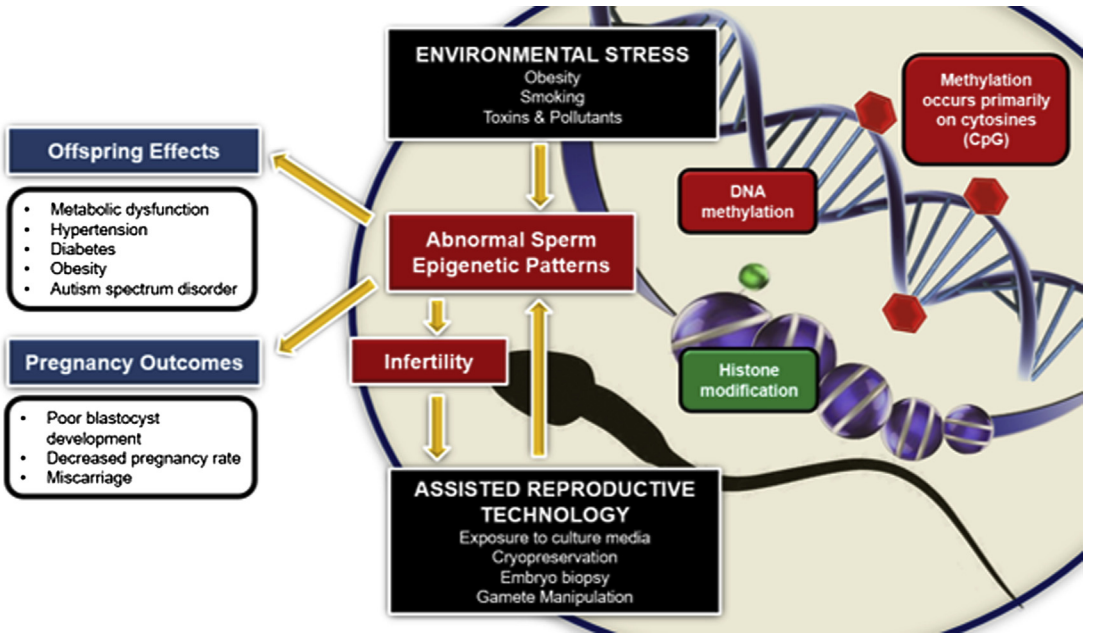


**Fig. 1.** The relationship between sperm epigenetic changes and assisted reproductive technology in patients with male factor infertility.

with male infertility phenotypes.[18] The results of these efforts have consistently demonstrated altered sperm acetylation and methylation patterns among men with oligozoospermia and oligoasthenoteratozoospermia when compared with normozoospermic controls.[16,20,21]

The relationship between epigenetics and infertility has also become a topic of public interest. A 2017 systematic review and meta-analysis received a great deal of media attention after authors reported a 50% to 60% decline in sperm counts among men in North America, Europe, Australia, and New Zealand between 1973 and 2011.[22] Although this downward trend in semen analysis parameters is likely multifactorial or affected by confounders, lifestyle factors and the epigenetic changes which arise from environmental exposures, such as phthalates and bisphenol A are believed to contribute to the reported reduction in male fertility over the past several decades.

Because of the intrinsic link between male epigenetic markers and infertility, researchers have begun to investigate the potential use of the sperm epigenome as a prognostic tool for infertile couples.[23] Currently, validation studies are underway to assess the accuracy of algorithms, which have been developed with the goal of predicting fertility outcomes based on methylation array data from sperm.[18] Predictive algorithms related to the sperm epigenome may have practical benefits because studies have demonstrated that epigenetic aberrations in men may adversely affect early embryonic development.[23,24] Therefore, it is important to consider the possibility that men with epigenetic damage may experience diminished success with the use of ART as well as a potentially increased incidence of recurrent implantation failure or early pregnancy loss.

Although epigenetic changes may lead to diminished fertility, it has also been suggested that the use of ART per se can induce epigenetic changes, which may have detrimental effects on pregnancy outcomes and the health of offspring.[25–27] Potential mechanisms by which IVF may lead to epigenetic changes include gamete handling, embryonic exposure to culture media, cryopreservation, and procedures, such as ICSI or trophectoderm biopsy for preimplantation genetic testing.[28,29] Theoretically, epigenetic changes arising from ART may also manifest as health consequences in future generations. Researchers have analyzed CpG sites within gene promoters of the placenta and umbilical cord in children conceived spontaneously and those conceived through IVF. These studies have shown that children conceived via IVF or ICSI possess epigenetic alterations in genes involved in disorders, such as obesity, type II diabetes, hypertension, cardiovascular function, and delayed growth velocity.[17,25–27] Although evidence exists supporting the idea that epigenetic changes arise from ART techniques, it is also important to consider the possibility that intrinsic maternal or paternal factors related to subfertility may be the true underlying cause of epigenetic abnormalities found in offspring achieved through ART.[27]

In summary, epigenetic dysregulation that results in male factor infertility or which potentially arises from gamete manipulation and ART may also impact the health of future generations.[30] Environmental exposures that alter epigenetic programming within the paternal germline may also transmit epigenetically altered patterns and phenotypes to future generations, even in the absence of ongoing environmental exposures.[30,31] Going forward, a clearer understanding of epigenetics is necessary to determine whether a true causal relationship exists between ART and epigenetic change. If such a relationship does exist, then optimization of IVF protocols to minimize the inheritance of epigenetic abnormalities should be an area of focus.

## SINGLE-NUCLEOTIDE POLYMORPHISMS AND COPY NUMBER VARIANTS ASSOCIATED WITH MALE INFERTILITY

Multiple genetic causes of male factor infertility have been proposed. However, publications evaluating genetic etiologies of infertility have produced conflicting results. Studies have explored the possible relationship between autosomal genes, single-nucleotide polymorphisms (SNPs), copy number variants (CNVs) and their potential impact on spermatogenesis and ART outcomes.[32–34] Although accumulating data support the important role of SNPs and CNVs in spermatogenesis, the effect of these variations on IVF outcomes remains to be determined and relatively few studies have investigated this subject.

A 2009 Dutch study investigated the relationship between infertility and single nucleotide changes in the genes NXF2, USP26, and TAF7L because these genes are believed to be crucial for spermatogenesis. Five autosomal genes (SYCP3, MSH4, DNMT3L, STRA8, and ETV5) were also evaluated. It was determined that changes in STRA8 and ETV5 were detected in a population of infertile men but not in a control group of men with normozoospermia. However, no other changes seemed to be linked to male infertility. Although the significant findings involving STRA8 and ETV5 were initially promising, a subsequent

functional analysis revealed that alterations in these genes (as well as in the other genes assessed) were unlikely to cause infertility in men.[32]

A 2012 study evaluated the possible association of 9 SNPs located on 8 different genes (FASLG, JMJDIA, LOC203413, TEX15, BRDT, OR2W3, INSR, and TAS2R38) with male infertility.[33] Using multiplex polymerase chain reaction/SNaPshot analyses followed by capillary electrophoresis, the study authors found that 3 of the 9 SNPs were significantly associated with male infertility (rs5911500 in LOC203413, rs3088232 in BRDT, and rs11204546 in OR2W3).[33] However, a 2017 case-control study failed to demonstrate any reliable associations between the TP53 gene and male infertility.[35] Similarly, an SNP of rs4880 of the SOD2 gene was found to have no association with male infertility in a study of 519 men with idiopathic infertility and 338 fertile controls.[36] Taken as a whole, it seems that although some SNPs have shown potential associations with infertility, others have not, and each individual SNP is unlikely to contribute in a significant fashion to male factor infertility in the larger sense. One of the major challenges with establishing associations between SNPs and infertility is that thousands or even tens of thousands of cases and controls would be required to generate strong conclusions.[37] The feasibility of conducting this type of large-scale research has limited the current understanding of this topic.

CNVs within specific genes have also been proposed as a cause of male infertility. A 2019 publication reported that CNVs in cation channel of sperm (CATSPER) genes are associated with idiopathic male infertility in the setting of normal semen parameters.[38] The application of array comparative genomic hybridization has been used to demonstrate that an increased number of specific distributions of CNVs may result in defective recombination and meiotic dysregulation. CNVs may also result in altered gene transcription and protein functioning, ultimately contributing to spermatogenic failure.[39]

It is highly likely that genetic mutations play a role in male fertility, but each individual polymorphism may only contribute to a small percentage of male infertility cases. Because of this, testing for SNPs in the general infertile population has not gained clinical applicability. In the future, it may be important to identify specific genetic alterations within the infertile male population because certain genetic etiologies of infertility may affect prognosis or outcomes with ART. Currently, there is insufficient data linking SNPs or CNVs to ART outcomes because the power to detect these associations requires extremely large numbers of patients.[37] The development of datasets incorporating genome-wide information from multiple institutions will likely be necessary to answer the important questions regarding the relationship between SNPs, CNVs, and clinical outcomes with IVF.

## DNA DAMAGE

Traditionally, the semen analysis has been the cornerstone of a male fertility evaluation. Despite its widespread use, routine semen analysis cannot measure the fertilizing potential of spermatozoa, and semen analysis parameters do not account for functional sperm characteristics.[40] Therefore, there has been a high level of interest related to the development of accurate tests, which predict sperm function and a semen sample's ability to achieve pregnancy. The level of sperm DNA damage has been studied with the goal of increasing the diagnostic sophistication and predictive value of tests before IVF and ICSI.[41] Incomplete apoptosis, the posttesticular environment, reactive oxygen species (ROS), and prolonged periods of abstinence are all proposed mechanisms by which sperm DNA damage may occur.[41] Reported associations between DNA damage and diminished reproductive outcomes has led to the use of sperm DNA integrity testing in many clinical practices.[42]

There are a variety of assays that can measure sperm DNA damage, including the single-cell gel electrophoresis (Comet) assay, the sperm chromatin dispersion (SCD) assay, the sperm chromatin structure assay (SCSA), and the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL) assay (**Table 1**).[41] Although each test possesses inherent advantages and disadvantages, TUNEL is arguably the most variable and has been difficult to standardize, although recently the TUNEL assay using a benchtop flow cytometer has been standardized and validated.[43,44] Both the SCD and SCSA methods are indirect assays, which only detect single-stranded DNA breaks and involve acid denaturation. The Comet assay is labor intensive, requires a fresh semen sample, and lacks a standardized protocol.[41] Unfortunately, to date, a perfect test does not exist, and the correlation between sperm DNA fragmentation and clinical outcomes remains somewhat questionable.

Traditional medical thinking as it relates to sperm DNA damage supported the idea that the epididymal environment protected spermatozoa and promoted the maturation of sperm. However, animal studies from the early 2000s contradicted these beliefs by reporting higher levels of DNA

**Table 1**
**Comparison of sperm DNA fragmentation assays**

| Assay | Type of Assay | Year Introduced | DNA Breaks Detected | Commercial Assay Available? | Specimen Type | Advantages | Disadvantages |
|---|---|---|---|---|---|---|---|
| SCD | Indirect | 2003 | Single-stranded DNA (ssDNA) | Yes | Fresh or frozen | • Does not rely on color or fluorescence intensity<br>• Does not require flow cytometer<br>• Simple, fast, reproducible, low cost<br>• Does not require complex instruments<br>• Standardized threshold values | • Involves acid denaturation |
| SCSA | Indirect | 1980 | ssDNA | No | Fresh or frozen | • Extensive body of literature<br>• Established clinical thresholds for results<br>• Reproducible with low coefficients of variation<br>• Rapid results | • Involves acid denaturation<br>• Relies on flow cytometry and fluorescence<br>• Relatively expensive<br>• Labor intensive<br>• Requires complex equipment |
| Comet gel electrophoresis | Direct | 1998 | ssDNA double-stranded DNA (dsDNA) | No | Fresh | • Can assess DNA in single cells<br>• Relatively inexpensive<br>• Does not require flow cytometer | • Time and labor intensive<br>• No standardized protocol<br>• Requires viable single-cell suspension<br>• Does not provide information on DNA fragment size |

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**Table 1**
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| Assay | Type of Assay | Year Introduced | DNA Breaks Detected | Commercial Assay Available? | Specimen Type | Advantages | Disadvantages |
|---|---|---|---|---|---|---|---|
| TUNEL | Direct | 1993 | ssDNA<br>dsDNA | Yes | Fresh or frozen | • Recently standardized and validated with benchtop flow cytometer<br>• Can make assessment with low numbers of sperm<br>• Can distinguish individual cells | • More expensive than other methods<br>• High intra-assay and interlaboratory variability |

damage and decreased fertilization rates in sperm harvested from the epididymis or ejaculate compared with surgically extracted sperm from the testicle itself.[45] The role of the epididymis in sperm DNA fragmentation was further investigated by Gawecka and colleagues,[46] who reported that fluid from within the epididymis and vas deferens activates sperm chromatin fragmentation in a murine model.

In humans, publications have demonstrated lower levels of DNA fragmentation in testicular sperm, and some authors have documented higher live birth rates with ICSI in patients who used testicular sperm as opposed to ejaculated sperm.[47,48] However, the exact etiology of sperm DNA damage remains unknown, and studies comparing reproductive outcomes with epididymal and testicular sperm are contradictory and inconclusive.[41] Despite a body of evidence supporting the epididymis as the site where DNA damage accrues in spermatozoa, human studies have failed to demonstrate superiority of testicular sperm to produce higher fertilization rates or live birth rates.[49,50] A 2018 meta-analysis demonstrated lower clinical pregnancy rates and fewer high-quality embryos in patients with high degrees of DNA fragmentation, but no significant difference in live birth rates.[51] This meta-analysis also highlighted one of the primary limitations with current research regarding associations between sperm DNA damage and pregnancy outcomes, specifically the heterogeneity of studies and the use of multiple sperm DNA testing platforms, which often lack standardization.[47,51] Similarly, a meta-analysis from 2016 demonstrated a lack of predictive value for the TUNEL assay, SCD test, and Comet assay and reported no relationship between test results and IVF/ICSI outcomes.[52] Although damage to sperm DNA may certainly play a role in ART success and a couple's fertility potential, testing for sperm DNA fragmentation has not yet resulted in meaningful improvements in clinical outcomes.

## FRESH VERSUS FROZEN SPERM

Since the first published report of human sperm freezing in 1957, cryopreserved sperm has become an integral component of reproductive medicine and modern infertility practice.[53] In addition, the cryopreservation of sperm has become a standard way to bank gametes in oncology patients and in patients undergoing vasectomy. Cryopreservation is also an essential aspect of sperm donation programs. From a logistical standpoint, cryopreservation of sperm has many advantages. However, the use of fresh versus frozen sperm for fertilization in ART is an area of significant debate.

There is very little consensus within the literature regarding the impact of cryopreservation on reproductive outcomes after conventional IVF or ICSI.[54] When either fresh or cryopreserved sperm is used for fertilization, samples have most frequently been obtained from the ejaculate. Because of several patient factors, however, it is not uncommon for spermatozoa to be obtained from the testes. Studies have addressed the use of fresh compared with frozen sperm as well as ejaculated versus testicular sperm. Despite this relative abundance of research, results have been contradictory.[54,55]

Several publications have reported that cryopreservation does not detrimentally affect outcomes. For example, in 1996, Gil-Salom and colleagues[56] reported no difference in fertilization rate, cleavage rate, or embryo morphology when comparing cryopreserved and fresh testicular spermatozoa in a population of men undergoing ICSI. Similarly, Ben-Yosef and colleagues[57] in 1999 reported similar outcomes with fresh and cryopreserved sperm in men with nonobstructive azoospermia (NOA) undergoing testicular sperm extraction (TESE) procedures. The authors suggested that performance of TESE followed by sperm cryopreservation before the initiation of ovarian stimulation should be considered first line treatment and would allow for more adequate patient counseling based on TESE findings without sacrificing pregnancy outcomes. Publications evaluating the use of cryopreservation with ejaculated sperm have also demonstrated that cryopreservation of spermatozoa from men with poor sperm quality does not negatively affect fertilization and pregnancy rates after ICSI.[58]

Conversely, other studies have documented clear correlations between cryopreservation of sperm and diminished membrane integrity, viability, and motility.[59] The mechanical and osmotic stress associated with cryopreservation have also been linked to abnormal morphology, and an increase in ROS related to the freezing process has been reported to induce DNA fragmentation.[60] In a recent publication by Schachter-Safrai and colleagues,[54] it was determined that in cases of cryptozoospermia, frozen-thawed ejaculated sperm is inferior to fresh ejaculated sperm based on a comparison of fertilization rates. However, in men with NOA, no major differences were found between fresh and frozen-thawed testicular sperm.[61] A 2004 publication reported that in a population of men undergoing ICSI, cryopreservation of sperm resulted in higher fertilization rates but lower embryo quality, lower pregnancy rates,

and lower delivery rates.[62] Taken as a whole, the existing literature remains inconclusive.

During the cryopreservation process, cryoprotectant agents, such as glycerol, ethylene glycol, dimethyl sulfoxide and dimethylformamide are incorporated into freezing protocols to minimize damage to the spermatozoa during the freeze-thaw process.[63] Despite the use of cryoprotectants, the formation of intracellular ice crystals, toxicity related to the cryoprotectants themselves, and factors of osmotic, mechanical, and oxidative stress all contribute to loss in sperm motility, decreased survival during the thawing process, and aberrant intracellular calcium concentrations.[63]

Although vitrification is now the most frequently used method to store oocytes and embryos, this method has been difficult to use in spermatozoa due to the relatively high concentrations of permeable cryoprotectants required.[64] Recently, publications reporting novel vitrification protocols have shown improved sperm survival rates, higher motility, and lower levels of DNA fragmentation when compared with conventional slow freezing of sperm.[64,65] Use of alternative cryoprotectant agents, such as sucrose have also been proposed as a potential way to improve sperm motility, viability, and mitochondrial membrane potential integrity when coupled with vitrification.[64–66]

Despite continued controversy regarding potential differences in outcomes after the use of fresh or frozen sperm, optimization of vitrification techniques for sperm samples may prove to be important in clinical practice in the years to come.[64] Vitrification may ultimately result in improved semen parameters for cryopreserved specimens. In cases of severe male factor infertility, azoospermia, or in situations where only small numbers of spermatozoa are available for cryopreservation, vitrification could provide a viable alternative to conventional slow freezing. At present, semen analysis parameters from fresh specimens are generally superior to parameters using frozen sperm, although any long-term clinical advantages of fresh specimens over frozen remain to be determined.

## OBESITY AND SPERM EPIGENETICS

In the United States, the prevalence of obesity in men of reproductive age has tripled since the 1970s, currently affecting greater than 33% of the adult population.[67] Increasing rates of obesity have coincided with reports of decreased sperm quality and rising rates of male factor infertility.[22,68] The relationship between obesity and male factor infertility is multifactorial, but epigenetic alterations in sperm are thought to be induced by obesity and lifestyle. These epigenetic abnormalities may negatively affect embryogenesis and the health of offspring.[69]

In the context of obesity, epigenetic programming seems to be altered in men with raised body mass indices (**Fig. 2**). A 2016 publication by Soubry and colleagues[70] demonstrated that men who are overweight or obese exhibit traceable alterations within the sperm epigenome. Specifically, lower methylation percentages at the MEG3, NDN, SNRPN, and SGCE/PEG10 differentially methylated regions exist in obese men when compared with lean controls. The finding of alterations within imprinted genes and methylation abnormalities within male gametes provides a useful foundation for ongoing studies investigating the relationship between obesity and epigenetic changes.

Another publication by Donkin and colleagues[71] in 2015 highlighted the dynamic nature of the sperm epigenome in humans and reported how environmental pressures at various time points, including obesity and diet, play a role in the propagation of metabolic dysfunction to future generations. Donkin's publication described distinct small noncoding RNA profiles in the sperm from obese men, which differed from their lean counterparts. Children of obese men were also found to be at a higher risk of developing obesity, metabolic syndrome, diabetes, and autism spectrum disorder.[68,71] The mechanisms that contribute to sperm quality issues may result in metabolic disturbances in offspring that persist into adulthood.[69] Interestingly, the influence of weight loss after bariatric surgery on sperm DNA methylation profiles showed relative plasticity of the epigenome. After undergoing gastric bypass surgery, DNA methylation profiles from ejaculated sperm samples exhibited rapid remodeling of the sperm epigenome in as little as 1 week after surgery. Over the course of 1 year after surgery, men who had previously been obese exhibited high degrees of normalization of their sperm epigenetic profiles when weight loss was sustained.[71]

In addition to alterations within the sperm epigenome, male obesity has been linked to poorer ART outcomes. A systematic review and meta-analysis from 2015 reported that obese men are more likely to suffer from male factor infertility (odds ratio [OR] = 1.66; 95% CI, 1.53–1.79) and have lower live birth rates per IVF cycle (OR = 0.65; 95% CI, 0.44–0.97). They experience an increased risk of nonviable pregnancy, demonstrate increased rates of DNA fragmentation, and have higher rates of abnormal sperm morphology.[72] Interestingly, the use of "freeze-all" protocols and subsequent frozen embryo transfer cycles may mitigate some of the negative effects of obesity on ART outcomes. Recent data demonstrated that in frozen

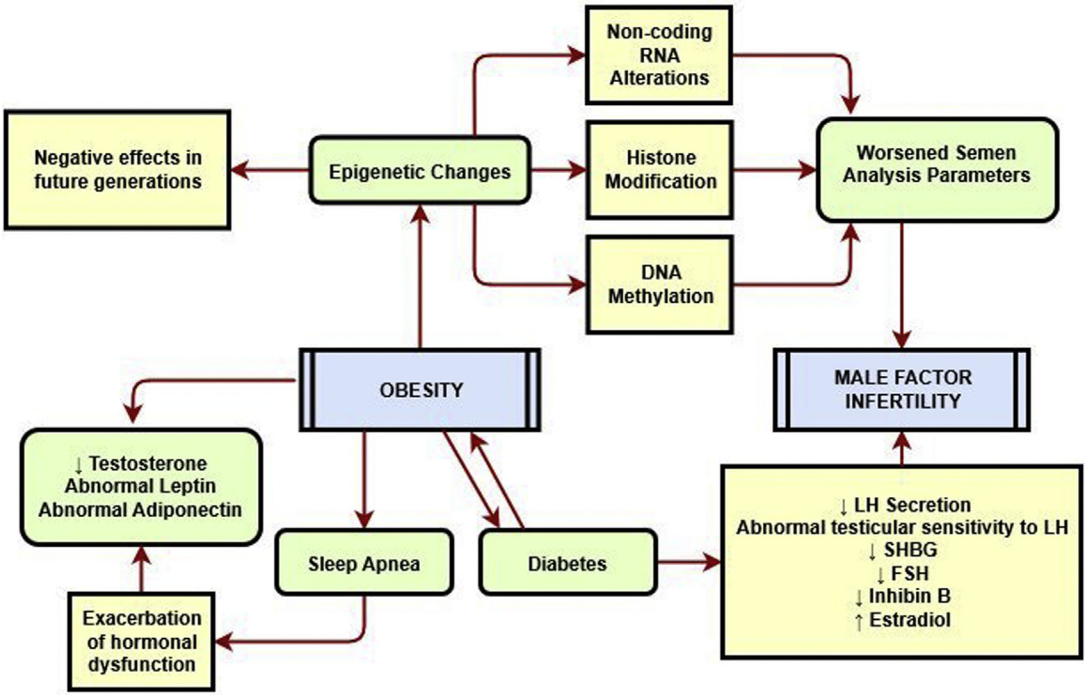


**Fig. 2.** The relationship between obesity and male factor infertility.

embryo transfer cycles after ICSI, raised body mass index and percent body fat determined by bioelectric impedance analysis did not negatively impact rates of fertilization, blastocyst formation, rates of euploidy, or sustained implantation.[73] Ongoing studies are necessary to further delineate the relationship between obesity and epigenetic changes. In the future, improvements in IVF outcomes may be realized if weight loss goals are met before initiation of fertility treatment.

## THE IMPACT OF PATERNAL AGE ON SPERM GENETICS AND PREGNANCY OUTCOMES

Similar to what has been observed in women over the past several decades, the decision to delay parenthood among men is becoming increasingly common.[74,75] The impact of advanced maternal age on fertilization and obstetric outcomes is well documented, with known associations between older female age and higher risk of infertility, spontaneous abortion, congenital anomalies, chromosomal abnormalities, and perinatal complications.[76] However, relatively few data are available regarding the role of advanced paternal age on fertility.

Of the studies available, some have shown no relationship between older male age and IVF outcomes, whereas others have reported abnormalities related to semen analysis parameters, sperm genetic integrity, and pregnancy outcomes.[75,77,78] Although the underlying mechanisms for adverse reproductive outcomes related to advancing male age are poorly delineated, researchers have proposed an increased incidence of sperm aneuploidy or increased sperm DNA fragmentation as potential causes.[77] Publications have also reported decreased testicular volume, a decreased number of functional Sertoli cells, abnormalities in testicular blood flow, endocrinopathies, and hypothalamic-pituitary-testicular dysfunction related to increasing male age.[76]

Spermatogenesis requires regular mitotic divisions of spermatogonial stem cells over the course of a man's reproductive life. As men age, the efficiency of their DNA repair mechanisms and their ability to defend tissues against ROS damage seem to decline.[79] As a result, de novo point mutations increase with advancing paternal age and may result in both rare and common genetic disorders. It has been estimated that somewhere between 1 and 3 de novo mutations are added to the germline mutational load of offspring for each additional year of paternal age at the time of conception.[79,80] Chromosomal abnormalities within sperm are typically the result of meiotic errors, which occur in early spermatogenesis. These meiotic errors can be related to either chromosome number (aneuploidy) or structural aberrations.[79] Abnormalities of the centrosome and epigenetic alterations in sperm

related to age can also alter fertility potential and embryo development for older men.[77,81]

Overall, the effect of older paternal age on IVF outcomes is mixed, and a strict definition of advanced paternal age does not exist. Studies that have demonstrated differences in outcomes have argued that after controlling for female age, older male age does affect pregnancy outcomes and blastocyst formation, although it is unclear whether all stages of embryo development are affected equally.[82] Many studies evaluating this issue have used the oocyte donor population as a way to indirectly reduce the impact of older female age and aneuploidy as confounders.[77] A 2015 systematic review evaluated the impact of paternal age on pregnancy and live birth rates in the setting of an oocyte donor model. This publication evaluated 12 studies incorporating 12,538 oocyte donation cases. The authors concluded that advancing paternal age is not associated with diminished pregnancy or live birth rates.[83]

Another way to decrease the confounding impact of maternal age is to study paternal age in euploid embryos, which have undergone preimplantation genetic testing. A 2017 study evaluating the relationship between paternal age and pregnancy outcomes in the setting of a single euploid embryo transfer determined that if a couple is able to generate and transfer a euploid embryo, there seems to be no difference in pregnancy outcomes (implantation rate, clinical pregnancy rate, and spontaneous abortion) between younger and older men.[77] Similarly, increased paternal age has been associated with decreased blastocyst formation and higher rates of aneuploidy, but in the setting of a single euploid embryo transfer, pregnancy outcomes do not seem to be negatively affected.[84] In a separate study, no associations were noted between advanced paternal age and embryology outcomes (fertilization rate, rate of blastocyst formation, euploid rate) or pregnancy outcomes (implantation rate, delivery rate, loss rate) when surgically extracted sperm was used for fertilization with ICSI.[85] Taken as a whole, it is plausible to presume that the male aging process has at least some detrimental impact on reproductive outcomes. However, the literature has not conclusively found this to be true, and numerous confounders related to this issue make definitive evidence difficult to obtain.

## MICROFLUIDIC DEVICES AND SPERM SELECTION

The issues presented previously in this article represent significant challenges to the success of ART in the setting of male factor infertility. To combat these challenges, new technologies have been investigated and applied clinically. One such advancement has been the use of microfluidic devices as a modality to process semen samples. This application has shown particular promise in patients with NOA. Simple swim up methods or density gradients have traditionally been used for semen processing before ART. More recently, microfluidic platforms have been proposed as a more effective way to select high-quality sperm by mimicking the in vivo process without centrifugation.[86] Microfluidic devices consist of small fluid-filled channels through which sperm are able to travel, more closely resembling the physiologic conditions of the female reproductive tract.[87] By avoiding mechanical damage related to centrifugation, microfluidic systems have been shown to select for spermatozoa with decreased levels of sperm DNA fragmentation.[88]

However, the value of microfluidic sperm sorting devices ultimately lies in their ability to select sperm, which will more effectively fertilize an oocyte. Unfortunately, improvements in ART outcomes have yet to be confirmed with microfluidics. A recent sibling oocyte study published in 2019 demonstrated that sperm sorting with a microfluidic chip does not significantly improve embryo kinetics or pregnancy outcomes after ICSI.[89] Similarly, fertilization and pregnancy rates were found to be no different when comparing density gradient versus microfluidic processing techniques in a population of patients with prior failed fertilization. It should be noted that the lack of difference in clinical outcomes occurred despite improvements in sperm DNA fragmentation indices with microfluidics.[90]

Although there is a lack of convincing evidence that pregnancy outcomes are improved with the use of microfluidic processing, this modality possesses several potential benefits. Microfluidic technology essentially automates a selection process, which previously required significant intervention.[91] Microfluidics allows for the relatively simple selection of a single sperm based on both motility and morphologic characteristics. Furthermore, the microfluidic chip devices are compact, portable, and straightforward to implement in the clinical laboratory.[91] This technique also reduces the mechanical stress placed on gametes, minimizes interoperator variability related to sperm processing, and has the potential to decrease costs associated with time-intensive laboratory procedures.[92]

The future of sperm selection techniques may rely heavily on advancements in single sperm diagnostics and the isolation of spermatozoa with the highest fertilizing potential. Microfluidic platforms

have allowed for isolation, manipulation, and analysis of single sperm cells. This ability is particularly useful in cases of small volume samples or cryptozoospermia.[93] Although many microfluidics devices separate sperm based on motility, men who undergo surgical sperm extraction via TESE pose a clinical dilemma because many viable sperm cells obtained surgically lack motility. Building on microfluidics principles and applying strategies, such as microscale filters, fractionated flow, dielectrophoresis, inertial microfluidics, hydrodynamic filtration, and deterministic lateral displacement may allow for appropriate isolation of healthy sperm in surgical specimens going forward.[94] If microfluidic cell separation devices can be fabricated which successfully isolate nonmotile sperm for use in fertilization, that would represent a significant advancement for men with NOA or those who require surgical sperm extraction.

## SUMMARY

The relationship between a man's overall health, male factor infertility, and ART outcomes are areas of ongoing research. At present, there is strong evidence that epigenetic changes within the male germline are prevalent in men with infertility. Through transgenerational inheritance, alterations in epigenetic patterns may also have consequences for the offspring of infertile men. Nevertheless, it remains to be seen whether the ART process or underlying differences inherent to the infertile male population contribute significantly to long-term outcomes. Numerous genetic factors are also known be involved in proper functioning of the male reproductive system, although the relative contribution of individual genetic mutations to male factor infertility as a whole is likely insignificant. Sperm DNA damage, sperm cryopreservation techniques, obesity, paternal age, and countless other factors likely contribute to a man's success rates with fertility treatment. Going forward, as associations between specific factors and ART outcomes become clearer, researchers and physicians will hopefully be able to individualize fertility treatments for men to optimize outcomes based on specific risk factors and the underlying cause of infertility. In summary, it is clear that the male contribution to ART success is significant, and a better understanding of these issues will hopefully result in improved outcomes in the future.

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