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Review Article

A Comprehensive Review on Male Infertility

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Abstract

The failure to attain a clinical pregnancy naturally after one year of unprotected intercourse is termed as infertility. Some of the factors that contribute to Infertility are late marriages, advanced age, genetics and lifestyle to name a few. To tackle this scenario, infertile and sub fertile couples take the aid of assisted reproductive technology. Intra uterine insemination (IUI), In vitro fertilization (IVF), Intra cytoplasmic sperm injection (ICSI), surgical sperm retrieval are commonly used procedures in ART. The exposure of chemicals which may contain heavy metals in working environment can cause sperm DNA fragmentation. The fertilization of an oocyte with high DNA fragmentation index may affect the clinical outcomes such as poor implantation or even miscarriage. In this review, we attempt to uncover how endocrine disrupting chemicals affect the sperm as well as some strategies to be considered while selecting a sperm for ICSI and subsequently assure a better clinical outcome.

Keywords: Heavy Metals; DNA Fragmentation; ICSI; Biomarkers

Introduction

The failure to attain a clinical pregnancy after one year of unprotected intercourse is defined as infertility [11,14]. About 10–15% of couples in their reproductive age suffer from infertility [1,11,14]. More than 186 million people suffer from infertility indicating it as a major health issue across the globe [7]. 30% of infertility cases is caused by male factors alone whereas male and female factors together contributes to nearly 50% of infertility [7]. To increase the fertilization success rates, medical assisted reproductive techniques such as intrauterine insemination, *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI), requires electing the best quality sperm from semen samples [7].

The site of spermatogenesis is seminiferous tubules of the testes, where germ cells undergo differentiation to form mature spermatozoa. Mitotic phase, meiotic phase, and the post-meiotic phase are the three distinguishable phases involved in the spermatogenesis process [28]. Histone proteins facilitates the DNA organization in all eukaryotic organisms through chromatin association [28]. Even when sperm parameters fall within the normal range, sperm cells may still exhibit significant DNA damage while maintaining the capacity to fertilize an oocyte [11,18].

The relationship between tobacco smoke and the prevalence of cardiovascular diseases and cancer is entrenched; furthermore, a research study by [31]. has accentuated the detrimental effects of tobacco smoking on male fertility. Erectile dysfunction, varicocele, congenital dysplasia, endocrine disorders, immune factors, sexually transmitted infections, and exposure to chemicals and radiation are some of the major risk factors associated with male infertility [31]. Tobacco smoking can reduce sperm concentration by interfering many pathways, such as the impairment of hormone and enzyme production which are critical for spermatogenesis as well as cause histological damage to reproductive organs [31]. The societal perspective of late marriages and family planning has now become a major factor that contributes to huge number of individuals seeking fertility treatments globally. Extensive use of ART treatments continues to accelerate every year [29].

Even though semen analysis plays a pivotal role in evaluating male infertility, it has intrinsic limitations. Surgical sperm retrieval is opted for infertile men who are diagnosed with azoospermia, a condition characterized by the absence of sperm [13]. The absence of sperm in ejaculate even after high speed centrifugation of semen sample confirmed by examining the sample at least twice is called Azoospermia. About 10-15% of infertile men suffer from azoospermia [33]. Azoospermia can be of two types – obstructive azoospermia and non-obstructive azoospermia. The absence of sperm in the ejaculate fluid caused by a physical blockage or obstruction within the male reproductive tract such as epididymis, vas deferens, or ejaculatory ducts is called obstructive azoospermia [34]. Percutaneous epididymal aspiration (PESA), microsurgical epididymal sperm aspiration (MESA) or open testicular biopsy are the surgical sperm retrieval procedures opted for patients diagnosed with obstructive azoospermia [32]. As compared to obstructive azoospermia, fertilization and clinical pregnancy rates is lower in non-obstructive azoospermia [33].

Endocrine-disrupting chemicals (EDCs)

Endocrine-disrupting chemicals (EDCs) can hamper the endocrine functions at both the receptor and cellular levels by imitating as natural hormones with an agonistic effect or impede their actions through antagonistic effects and even intervene to disrupt the metabolic processes (1). Furthermore, environmental endocrine disruptors can interfere with the hypothalamic-pituitary-gonadal (HPG) axis through autophagy, as indicated by [6]. Substances resulting from the incomplete combustion of organic materials may have the potential to disrupt endocrine functions. The activation of factors that promote sperm apoptosis correlates with an increase in the sperm DNA fragmentation index (DFI) [8]. Mining, smelting, and agricultural practices are some of the industrial activities that caused the extensive pollution in many parts of the world [2]. Lead (Pb), aluminum (Al), mercury (Hg), arsenic (As), and cadmium (Cd), are some of the identified heavy metals that generate toxic effects mediated through biomolecules such as proteins and DNA and play critical role in structural, catalytic, or transport functions [2].

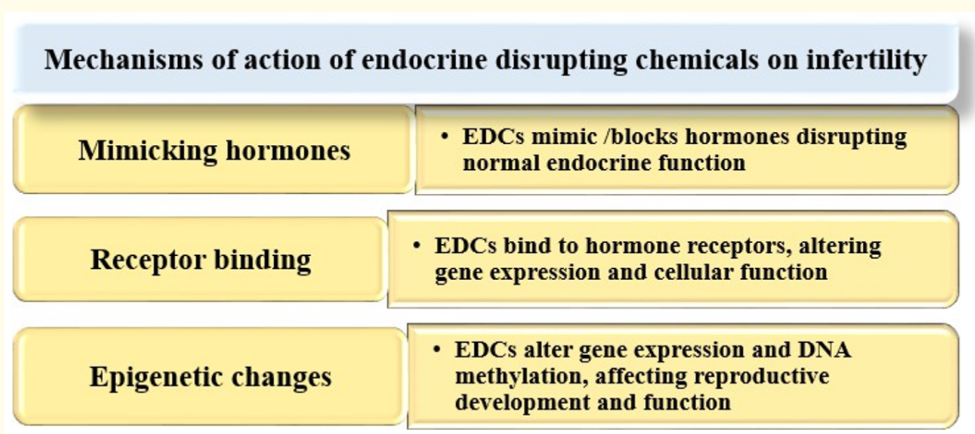

Figure 1: Mechanisms of action of endocrine disrupting chemicals in infertility.

Tobacco smoking diminishes sperm concentration, which may occur due to impaired hormone and enzyme production that affects spermatogenesis, as well as histological damage to reproductive organs. Tobacco smoke acts as an endocrine disruptor [31]. Moreover, tobacco smoke is associated with irregular protein expression and both genetic and epigenetic alterations in spermatozoa [31]. Consuming cigarettes containing cadmium (Cd) has been interrelated with reduced testicular size, which in turn manifest as low sperm count and is also lead to the apoptosis of sperm cells [2]. *In vitro* studies conducted on animals showed that Cd adversely affects gonadal development [2]. Cadmium (Cd) has been shown to reduce serum levels of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. In mature male offspring, arsenic exposure leads to autophagic changes and mitochondrial dysfunction within the hypothalamic-pituitary-gonadal (HPG) axis and sperm function, mediated through AMPK/tuberous sclerosis complex (TSC)/mTOR and LC3-related pathways [6].

Mercury exposure leads to DNA breaks in spermatozoa, resulting in low sperm motility, dysfunction, and viability according to some *in vitro* studies [2]. Lead toxicity is characterized by the generation of reactive oxygen species (ROS), the displacement of zinc in metallothionein (MT), which affects zinc bioavailability, and the disruption of the blood-testis barrier through the substitution of calcium in the zona adherens junction [2]. Epidemiological studies indicates arsenic exposure in humans lead to male reproductive dysfunction, such as decreased testicular weight, reduced accessory sex organ weight, impaired sperm viability and motility, lower epididymal sperm counts, and low levels of gonadotrophins and testosterone, along with reduced steroidogenesis [2].

The hazardous compounds present in environment has the ability to supress the production of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase which in turn increases oxidative stress [15]. Reactive oxygen species are produced when pollutants come in contact with the human body, which then causes oxidant injury and pro-inflammatory effects. The impairment of ATP synthesis happens due to the mitochondrial damage [15]. Mitochondria serves as energy source in oocyte maturation, fertilization, and early embryonic development by aiding in transcription and translation processes [19]. A lot of metabolic, epigenetic, redox, and calcium signalling processes that are essential for cellular function are regulated by mitochondria [19]. Many upcoming studies indicates that mitochondrial dysfunction leads to oocyte ageing [19].

Biomarkers influencing sperm function and viability

Sperm DNA integrity plays a significant role in normal fertilisation, implantation, pregnancy, and foetal development [3,26]. In the post-meiotic phase of spermatogenesis, known as spermiogenesis, histones are substituted with transition nuclear proteins and protamines [10]. Protamines, which are basic proteins abundant in arginine and cysteine, play a pivotal role in the final packaging of DNA [10]. Abnormalities in protamine composition have been associated with male infertility [10]. Low levels of protamines, the ratio of protamines 1 to 2, the number of cysteine groups in protamine 1, and the replacement of histones by protamines can lead to DNA fragmentation [10]. Consequently, this fragmentation may result in sperm morphological abnormalities, low motility, reduced fertilization rates, or even mutations that jeopardize offspring viability [10].

A specific condition known as multiple morphological abnormalities of the flagella (MMAF) is identified by the absence of flagella or the presence of bent and coiled flagella [24]. Genes related to spermatogenesis or ciliogenesis such as DNAH1, AKAP4, CAFP43, and CAFP44 are responsible for inducing MMAF in humans [24]. According to [9], DNAH8 has emerged as a potential biomarker for asthenoteratozoospermia. [9] propose that proteins associated with dynein arms may influence spermatogenesis, particularly due to the impact of DNAH8 deficiency on the localization of DNAH17 within sperm flagella. Intracytoplasmic sperm injection (ICSI) is presented as a viable fertility treatment for cases of male infertility linked to DNAH8-associated MMAF [9]. Autophagy- related proteins, including LC3, ATG5, ATG16, BECN1, p62, mTOR, AMPKa 1/2, and PINK1, along with their upstream regulators, exhibit functional activity in human spermatozoa, indicating a potential role for autophagy in the regulation of sperm motility, as noted by [6]. Furthermore, autophagy plays a critical role in various processes during spermiogenesis, including acrosome biogenesis, flagella assembly, head shaping, and the elimination of cytoplasmic remnants from elongating spermatids [6].

DNA fragmentation

Sperm DNA integrity is measured by analysing the Sperm DNA Fragmentation Index (DFI) [12]. Ensuring the integrity of DNA is vital for appropriate embryonic development. When DNA is compromised, it can obstruct the embryogenesis process, which may lead to early miscarriages or produce offspring with notable birth defects (1). During the maturation phase, sperm chromatin becomes highly condensed; however, irregularities in protamine processing or the failure of certain germ cells to undergo apoptosis can lead to the production of defective mature sperm, often characterized by an increased sperm DNA fragmentation index (DFI). Environmental factors, particularly exposure to endocrine disruptors, may compromise the integrity of the sperm genome, especially within the sperm nucleus, where DNA is loosely associated with histones in the nucleohistone compartment [31]. DNA fragmentation typically arises during the later stages of spermatogenesis due to deficiencies in DNA repair mechanisms, which may be influenced by various pathological factors such as apoptosis, increased oxidative stress from elevated levels of reactive oxygen species and imbalances in chromatin components like protamines and histones [26]. Contributing factors to these alterations may include substance abuse, tobacco use, environmental contaminants, elevated testicular temperatures, and advancing age [26].

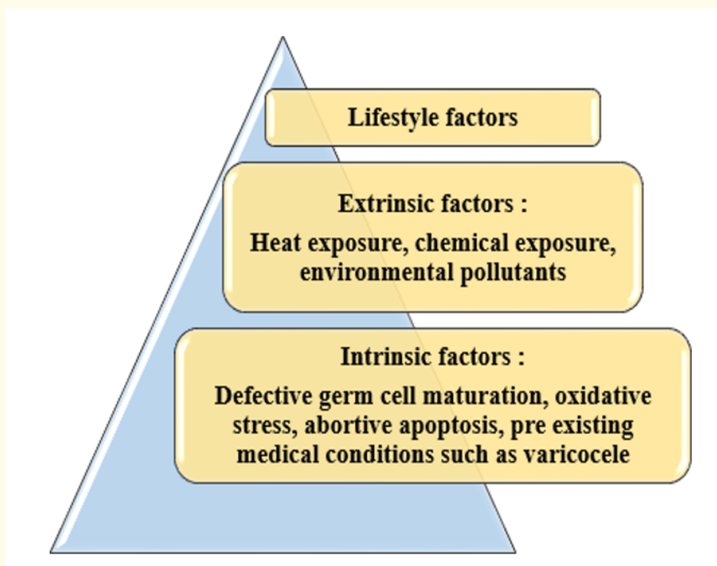

Figure 2: Factors affecting sperm DNA fragmentation.

Sperm DFI has a notable impact on live birth rates, the quality of embryos, and the incidence of miscarriages [8]. The implications of sperm DNA fragmentation in the context of *in vitro* fertilization and embryo transfer remain a subject of ongoing debate [8]. The investigation into the relationship between sperm DNA and miscarriage has only recently gained attention [4]. Damage to the male genome is a significant factor associated with embryo non-viability. The integrity of DNA in both gametes is essential for the successful development of an embryo to term [4]. The clinical ramifications of sperm DNA damage may manifest later, as the paternal genome has a more pronounced effect on the embryo during its later stages of development [4].

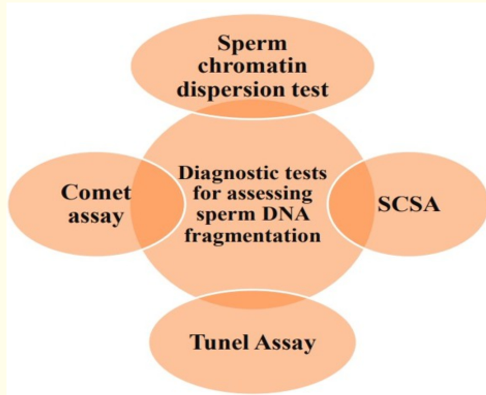

Figure 3: Diagnostic tests for assessing sperm DNA fragmentation.

The Sperm Chromatin Structure Assay (SCSA) is widely utilized for assessing DNA fragmentation due to its robustness, high precision, and minimal bias [11,18]. Nonetheless, it does not directly measure DNA strand breaks; instead, it evaluates the DNA's susceptibility to acid denaturation and cannot differentiate between single and double-stranded breaks [11,18]. Alternative assays, such as the Comet or TUNEL assays, provide a more direct measurement of DNA breaks [11,18]. Evidence suggests that men with unexplained infertility typically present with higher DFI levels [12].

Sperm selection for ART treatment

The techniques used in assisted reproductive technologies (ART) emerged from the foundations of scientific research on animal reproduction in the past few decades [L. B. Ferré., *et al*]. Numerous techniques are employed in assisted reproduction technologies to segregate the viable sperms from ejaculate. Swim-down, swim-up, migration-sedimentation, density gradient centrifugation, magnetic activated cell sorting, and glass wool filtration are some of the widely used sperm selection technique [21]. Magnetic activated cell sorting (MACS) is a very advanced and efficient technique used in ART that eliminates apoptotic cells from an ejaculate. The principle behind this is identifying phosphatidylserine residues on apoptotic sperm cells by annexin V conjugated superparamagnetic microbeads and separating the bound spermatozoa in a magnetic field [21]. The MACS sperm selection method is proven to be a phenomenal tool that delivers higher fertilization potential by selecting viable and morphologically normal sperm [21].

Now a days, the determining factor for an ART treatment is routine semen analysis, sperm count and motility in fresh ejaculate sample as well as after sperm preparation using gradient centrifugation and swim up [25]. The choice of ICSI as well as conventional IVF is also dependent on these factors [25]. Several studies have indicated that high sperm DNA fragmentation index (DFI) is inversely proportional to ART outcome [25]. The implementation of ICSI into clinical practice has remained as one of the prolific breakthroughs in the field of assisted reproductive technology (ART) since 1992 [17]. ICSI offers fertilization potential for patients suffering with severe male factors such as suspected azoospermia by surgical sperm retreival from epididymis or testicles unlike conventional IVF [17]. ICSI bypasses the natural selection since the sperms are selected by embryologist based on their motility and morphology [30].

Sperm selection represents a crucial phase in assisted reproductive technology (ART), typically relying on basic physical character-

SPERM SELECTION TECHNIQUES	CRITERIA OF SPERM SELECTION
Density gradient centrifugation	Morphology, Motility, Cell density
Swim up	Progressive motility
Density gradient centrifugation + Swim up	Morphology, Motility, Cell density
Microfluidic sperm sorting	Motility, Rheotactic, Chemotactic properties
Magnetic activated cell sorting	Removes sperms with apoptotic markers

Table 1: Commonly used sperm selection techniques.

istics such as morphology and motility [7]. However, this approach often overlooks the biochemical status of sperm, which is essential for successful fertilization and embryonic development, particularly in cases of severe asthenozoospermia where sperm exhibit minimal motility [7]. However, sperm that demonstrate favorable morphology and motility may still possess limited potential in the biochemical aspects of fertilization and blastocyst development [7]. In general, existing sperm selection techniques, whether traditional or microfluidic, remain confined to basic physical attributes such as morphology and motility. This limitation presents significant challenges for individuals suffering from severe and total asthenozoospermia, as their sperm are nearly immotile; consequently, current methodologies are unable to select sperm based on motility and, more critically, cannot ascertain which sperm are viable [7]. In these scenarios, embryologists are compelled to make selections based solely on morphology, leading to a fertilization success rate of merely 10%–20%, which is substantially lower than the 80% success rate observed with normal sperm samples [7]. Intracytoplasmic sperm injection (ICSI) has facilitated the utilization of highly teratozoospermic sperm samples in the context of *in vitro* fertilization (IVF) [5].

The application of assisted reproductive technology (ART) in reproductive medicine has a prolific impact and goes on with relevant and adequate treatments to help the infertile patients [35,36]. Recent studies have noted that, oocyte activation deficiency is caused by numerous PLCZ1 abnormalities in patients suffering from failed IVF and ICSI cycles. Ca2+ oscillations are induced by PLCZ1, which is responsible for oocyte activation. Numerous imperative cellular signalling pathways are governed by these calcium oscillations in early embryos [35,36]. Unexplained non male factor infertility can also be possible causes for IVF failure [35].

Numerous strategies have been proposed to improve sperm selection for intracytoplasmic sperm injection (ICSI). These strategies encompass factors such as the sperm's surface charge, the identification of non-apoptotic sperm, the utilization of hyaluronic acid (HA) for selection purposes, the nonadherence characteristics of sperm, and the assessment of motile sperm organelle morphology examination (MSOME). The zona pellucida (ZP) acts as the ultimate barrier for natural sperm selection, and ZP-binding techniques have been utilized to pinpoint appropriate sperm for ICSI. Mu., et al. have introduced the BLASTO-chip method, a non-invasive technique for sperm selection that utilizes metabolic profiles. This innovative method achieves over 90% accuracy in selecting viable sperm from samples containing immotile sperm, with successful fertilization and live births in mice demonstrating its biosafety [7].

Discussion

Reproduction is inevitable for humans to ensure the continuity and survival of our species [23]. Ovulation dysfunction, fallopian tube disease, and pelvic adhesion are the main reasons for female infertility whereas sexual dysfunction and defective spermatogenesis attributes to male infertility [23]. Medical assisted reproduction employs artificial insemination (AI), *in vitro* fertilization-embryo transfer (IVF-ET) and allied technologies to help infertile couples achieve pregnancy [16].

Recent research has increasingly indicated that autophagy is active in various mammalian organs and systems, including the embryo, placenta, liver, and both male and female reproductive systems, as reported by [6]. Autophagy, a catabolic process involving the degradation of cytoplasmic components within lysosomes, is vital for numerous physiological and pathological functions, as noted by [6]. To mitigate DNA damage caused by oxidative stress, adopting a healthy, unprocessed diet rich in antioxidants, along with antioxidant supplementation, may offer protective benefits; lifestyle improvements can enhance sperm quality within a timeframe of 3 to 6 months [4].

The increased production of reactive oxygen species (ROS) can lead to oxidative stress (OS), resulting in DNA damage and apoptosis of germ cells. Excessive levels of reactive oxygen species (ROS) can negatively impact male fertility by damaging various cellular components, which ultimately leads to sperm dysfunction. This detrimental effect includes lipid peroxidation and protein oxidation, mediated by diverse molecular pathways [31]. Heavy metals may interfere with male reproductive health through hormonal or genotoxic mechanisms, potentially breaching the blood- testis barrier and adversely affecting spermatogenesis, which is associated with decreased sperm motility and density, increased morphological defects, and subsequent male infertility [2]. The fertilization potential of sperm is hindered when DNA damage levels are high [27].

Regular exercise is recommended to maintain a healthy weight as well as improve fertility. Introducing meditation, yoga and deep breathing exercises into daily routine will reduce the stress and anxiety during the course of treatment. Implementing a healthy balanced diet rich in fruits, veggies, whole grains, proteins and antioxidants will improve fertility. An exemplary component to be included in diet is Quercetin, a powerful scavenger of oxygen free radicals [2]. This flavonoid is present in apples, onions, mulberries, potatoes, broccoli, tea, peanuts, soybeans, as well as red wine. This compound is a reservoir of many pharmacological properties, such as antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, anti-obesity, and reproductive system protective effects [2].

Conclusion

Infertility as well as subfertility arises due to many reasons. First and foremost thing that should be done by a couple is the detailed clinical diagnosis by a fertility specialist to get a comprehensive idea about the fertility quotient in both the partners. Along with treatment it is necessary to make certain changes in lifestyle. A healthy diet rich in anti-oxidants can reduce the raised sperm DNA fragmentation to a certain extent. Reducing the exposure to laptop, chemicals, fertilizers, cement etc. will help to retain the sperm dna integrity. Identifying novel molecular markers will aid the current ART treatment strategies to serve the patients even better. The advent of artificial intelligence has opened up a wide array of opportunities for scientists to explore reproductive medicine. Sperm selection plays an important role in the achieving success in an IVF cycle. Therefore many advanced sperm selection techniques have been discovered to help the embryologists pick the best viable and morphologically normal sperm. MACS, microfluidics, percoll density centrifugation, simple wash are widely used sperm preparation techniques that will yield us good quality sperms from the ejaculate sample. Surgical sperm retrieval procedures such as TESA, TESE, PESA, micro TESA etc. are used in cases of obstructive and non-obstructive azoospermia.

Conflict of Interests

The authors declare no conflict of interests.

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