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Male Infertility Problem: A Contemporary Review on Present Status and Future Perspective

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Abstract

Semen quality plays a pivotal role in maintaining healthy fertilizing ability of spermatozoa. Male infertility is a rising global problem with an increasing declining in male semen quality among men living in Africa, Europe, North American, and Asia. Though the sperm acquire proactive mechanisms during spermatogenesis and their epididymal maturation, they still remain viable for toxic insult. Declining semen quality is a major contributor to infertility. Studies have postulated that different factors, such as exposure to pesticides, industrial chemicals, heavy metals, obesity, alcoholism, tobacco smoking, sedentary lifestyles, poor nutrient intake, oxidative stress, physiological factors, genetic factors can influence male fertility. Routine semen analysis and assays for sperm chromatin integrity are the most widely utilized and best studied adjunctive diagnostics in male infertility. Over the years, scientists have developed different treatment options for male infertility. Male infertility with known etiology can be treated successfully, but other causes like genetic factors require pragmatic approaches. This article summarizes protective mechanisms of spermatogenesis, causes, diagnosis, and both modern and traditional treatment approaches of male infertility. Further, this article highlights present issues and direction for future exploration of the male infertility problem.

Keywords

male infertility, protective mechanisms, causes, diagnosis, treatment, future

Introduction

Infertility is a significant health problem affecting around 15% of couple. Most infertility is due to the male components of fertilization,[1] which are mainly due to male factors. Infertility is described as the inability to get pregnant during 12 consecutive months of unprotected intercourse.[2] By transmitting paternal genes to the egg, the spermatozoon (sperm), and specifically its nucleus, acts as a "genome bridge"[3] from one generation to another. Any deterioration or alteration of sperm chromatin may have serious consequences for either fertilization or the risk for developmental disorders in the embryo. Formed during spermatogenesis and epididymal maturation, the sperm nucleus is a uniquely condensed structure which has evolved to protect the haploid genome as it is carried to the oocyte.[4]

Recent discovery of declining trend of semen quality over past decades became a major concern. Retrospective evaluations of laboratory semen records are plentiful and have indicated a decrease in semen quality as reported from Belgium,[5] Finland,[6] France, Scotland,[7] Norway, the United Kingdom,[8] Greece,[9] Canada, and the United States.[10] In contrast, no variations have been reported from regions such as Denmark,[8] Israel,[11] Australia,[9] and Africa[12] But, the data reporting no variation in semen quality is still unconvincing considering the global trends. For example, it was recently confirmed that the Asian male population followed the same global trend over the period of past 50 years.[13]

Protective Mechanisms of the Sperm

Spermatogenesis

The formation of the sperm from a germ cell precursor, its maturation, transport, viability, and the final steps of

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fertilization are all complicated and sensitive processes. The production and formation of sperm occurs in the seminiferous tubules of the testis. Seminiferous tubules contain germ cells at various stages of development as well as the somatic cells known as Sertoli cells. One of the most important functions of the Sertoli cells is the formation of the "blood–testis barrier" or the BTB.[14] This is formed when adjacent Sertoli cells come into contact with each other and form a series of tight junctions thus regulating the passage of macromolecules from the systemic circulation to the developing germ cells.[15]

Since germ cells during meiosis may have chromatin decondensed for long periods (days), they are particularly susceptible to nuclear damage. Thus, the presence of a barrier may help to prevent noxious factors from reaching them. As germ cells undergo mitotic and meiotic divisions, several major types of cell can be distinguished in mammalian seminiferous tubules. These are the diploid stem germ cells (spermatogonia); germ cells after the first and second meiotic division (primary and secondary spermatocyte); developing haploid germ cells (spermatids) and testicular sperm.[16]

Spermatogonia that enter the spermatogenesis cycle will undergo a series of mitotic divisions and in turn divide meiotically into primary spermatocytes. One characteristic feature of male germ cells is that cytoplasm does not completely separate upon nuclear division.[17] As spermatocytes enter into the leptotene stage, they move away from the basement membrane toward the lumen of the seminiferous tubule. They are then separated from the basement membrane by the BTB. By now, since chromatin replication has already been occurring, these spermatocytes contain a tetraploid (4n) amount of DNA. Homologous chromosomes, one from each parent, will come together and nucleoli become more visible. At this stage, primary spermatocytes are known as zygotene spermatocytes. In the next stage (pachytene), the crossing over of chromosomes occurs, a process which is highly susceptible to chemical damage. The chromosome then become more condensed (diplotene). DNA synthesis is high in preleptotene spermatocytes while RNA synthesis is high in pachytene spermatocytes. At the final stage of meiosis, the chromosomes will then segregate from one another. This division results in the formation of secondary spermatocytes (2n). Finally, each one of the secondary spermatocytes complete the second phase of meiotic division to give rise to 2 haploid (1n) spermatids. DNA is synthesized during mitosis of each spermatogonia, while histones, the basic proteins surrounding the DNA, are produced in the nucleus at the inter phase of each cell cycle and are incorporated into newly synthesized DNA.[18]

Spermiogenesis

Spermiogenesis is a complex process that involves many morphological, physiological, and biochemical changes. These changes include nuclear condensation, shaping of the nucleus, formation of an acrosome, elimination of the cytoplasm, development of a flagellum, and the arrangement of mitochondria into the sperm middle piece. At the beginning of spermiogenesis, the nucleus contains decondensed chromatin and there is believed to be active transcription. But during the latter stages of spermiogenesis, the nucleus replaces lysine and histidine-rich histones with a series of basic proteins. Initially these are transitional proteins and ultimately arginine and cysteine-rich basic protamines. The spermatid becomes highly condensed. The replacement of nuclear histones by protamines and nuclear condensation during spermiogenesis involves a series of interactions mediated by the transition proteins. Two major classes of transition proteins TP1 and TP2 are found.[19]

As a result of this arrangement, mammalian sperm nuclear DNA becomes 6-fold more condensed than DNA of somatic cells (in an ordered process beginning at the anterior end of the nucleus and proceeding toward the tail. This makes sperm nuclear DNA, the most highly condensed eukaryotic DNA known.[20]

An important change to the sperm during epididymal transit is the formation of disulphide cross links in the nucleus. Protamine, rich in cysteine, contains sulfhydryl (S-H) groups that participate in the formation of covalent bonds[21] within and between the protamine molecules providing a highly stable keratinous nature. Production of the disulphide cross-links assures a stable condensation of the nuclear protein complex and makes the sperm nucleus uniquely resistant to sonication and strong detergents. These disulphide bonds are prominent only in the sperm of eutherian mammals and are particularly confined to the anterior region of the sperm nucleus. Nuclear stabilization of chromatin by disulphide bonds is a gradual process which is initiated in the first segment of the epididymis, the caput and is completed once sperm reach the cauda epididymidis.[22]

Human nuclear proteins contain a limited amount of unoxidized protamine and cysteine at the end of the epididymal passage. These free amino groups of arginine and free thiol groups of cysteine may be chelated by reversible binding of zinc ions, thus further condensing the sperm nucleus. It is thought that zinc in the sperm nucleus hinders premature thiol disulphide exchange and thereby decreases the vulnerability of sperm chromatin to chemical attack during transfer to ovum.[23]

Until ejaculation, storage of fully matured sperm takes place primarily in the cauda of the epididymis. Sperm can remain viable in the epididymis for about 4 to 6 weeks.[24] During this period of time, sperm could become exposed to toxicants.[25] If such chemicals subvert the normal condensation mechanism to protect the nucleus, they may have a detrimental effects on sperm nuclear integrity and function.[26]

Causes of Male Infertility

Environmental, occupational, and modifiable lifestyle factors may contribute to this decline of male fertility. Lifestyle factors associated with male infertility include smoking cigarettes, alcohol intake, use of illicit drugs, obesity, psychological stress, advanced paternal age, diet composition, and coffee consumption. Among other factors are testicular heat stress, intense cycling training, lack of sleep, and exposure to electromagnetic radiation from mobile phones.[27]

Environmental Chemicals and Other Factors in Impaired Spermatogenesis

In recent years, it was shown that the male reproductive tract is a potential site of toxic insult[28,29] to an increasing array of pollutants and chemicals in the environment. The rise in environmental pollution causes a significant increase in disease burden and costs in treating infertility disorders.[30]

Numerous toxicological studies in animals show that testicular tissue and spermatogenesis are vulnerable to numerous hazards because of the continuously ongoing large number of cell divisions with cell differentiation and maturation processes.[19] These hazards include not only environmental pollutants, such as industrial chemicals, heavy metals, or insecticides but also any occupational hazard including scrotal heat exposure. Thus, capillaries and Leydig cells in the interstitium are targets for cadmium and ethanol, respectively. Special targets in the seminiferous tubules are the Sertoli cells, spermatogonia spermatocytes, and spermatids. Even sperm epididymal maturation and sperm motility are directly affected.[18]

Organophosphorus Pesticide

Exposure of laboratory rats to very low doses of chlorpyrifos for 30 consecutive days caused significant reduction in testicular and epididymal weights.[31] Reduction in epididymal sperm count and sperm motility was evident in animals treated with 5 mg/kg and 10 mg/kg chlorpyrifos. Increased sperm abnormalities were observed after both doses, whereas increased DNA damages was observed even with the lowest dose of chlorpyrifos (2.5 mg/kg). Chlorpyrifos not only affected sperm parameters but also reduced serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels, which decreased fertility indices such as fewer of pregnancies, implantations, and live fetuses. Similar results were obtained with human spermatozoa indicating detrimental effects of organophosphorus pesticides to human spermatozoa.[32,33] Further, Martenies and Perry[34] documented a clear relationship between poor semen quality and environmental/occupational exposure to pesticides or their metabolites.

Industrial Chemicals

Industrial chemicals such as meta dinitrobenzene and methoxy acetic acid, which are plasticizers, can impair sperm fertilizing ability and sperm motility (measured using computer-assisted semen analysis) at both acute and chronic dose levels.[35,36] Exposure to industrial chemicals not only degrades male infertility but also can result in DNA fragmentation.[37]

Inorganic Pollutants

Metals toxicity depends on several factors, including their ability to bind to thiol groups of enzymes and proteins, thus altering their structure and/or function.[18] In particular, genotoxic elements (arsenic, cadmium, and nickel) may damage the DNA structure either directly through the production of oxygen free radicals or indirectly via the alteration of enzymes responsible for DNA repair.[38]

Lifestyle Factors

Tobacco smoking is responsible for DNA damage and the formation of reactive oxygen species. A study on the male partners of couples facing primary infertility found that teratozoospermia (abnormal morphology) was present in 63% and 72% of males who drank alcohol moderately (40-80 g/d) and heavily (>80 g/d), respectively. None of the heavy alcohol drinkers were normozoospermic (normal sperm) and most (64%) were oligozoospermic (low sperm count), which is suggestive of progressive testicular damage in relation to increasing daily alcohol intake.[39] Recreational drugs such as marijuana, cocaine, anabolic–androgenic steroids, opiates (narcotics), and methamphetamines are examples of illicit drugs that exert a negative impact on male fertility. The adverse effects of these drugs could impair the hypothalamic–pituitary–gonadal (HPG) axis, testicular architecture, and sperm function.[40]

More than 76% of caffeine consumers (3.0 ± 1.8 cups of coffee/d) had a slight increase in semen volume, whereas fertile vasectomy patients who drank 6 cups of coffee/d presented with higher sperm motility. A recent systematic review involving 19 967 men found that in most of the studies, semen parameters were affected by cola-containing beverages and caffeine-containing soft drinks, but not by caffeine intake from coffee, tea, and cocoa drinks. Caffeine intake may impair male reproductive function possibly through sperm DNA damage.[41]

A study involving 13 077 men reported that obese men were more likely to be oligozoospermic or azoospermic compared to men within a normal weight range. A population-based study found that as body mass index and waist circumference increased, the prevalence of low ejaculate volume, sperm concentration, and total sperm count were also greater in overweight and obese men of unknown fertility.[42] The presence of excess white adipose tissue in obese individuals causes increased conversion of testosterone to estrogen, and affects the HPG axis leading to a reduction in gonadotrophin release, and impaired spermatogenesis and increased oxidative stress.[43] Similarly, diet such as vegetables and fruits, fish and poultry, cereals, and low-fat dairy products were among the foods positively associated with sperm quality. However, diets consisting of processed meat, full-fat dairy products, alcohol, coffee, and sugar-sweetened beverages were associated with poor semen quality and lower fecundity rates.[44]

Stress, in its many forms, may be detrimental to male reproductive potential. The classical stress response activates the sympathetic nervous system and involves the hypothalamus–pituitary–adrenal (HPA) axis. Both the HPA axis and gonadotrophin-inhibitory hormone exert an inhibitory effect on the HPG axis and testicular Leydig cells. Men who were significantly stressed had lower levels of testosterone and

higher levels of FSH and LH than men with normal well-being thus reducing sperm counts and sperm morphology and motility.[45] Further, sleep disturbances may possibly have adverse effects on male fertility, as semen volume was lower in patients with difficulty in initiating sleep, including those who smoked or were overweight.[46]

Treatments

Semen analysis and assays for sperm chromatin integrity are the most widely utilized and best studied adjunctive diagnostics in male infertility. Sperm DNA fragmentation detects a high level of defective spermatozoa.[37] DNA damage is more common in infertile men than fertile men. If sperm count is less than 40 million, artificial insemination can be recommended. However, if the sperm count is less than 20 million, the following treatments are recommended:

Assisted Reproductive Technology

If the sperm counts are less than 20 million but have reasonable motility, in vitro fertilization (IVF) can be carried out. The introduction of IVF in 1978 created a comprehensive shift in the focus of reproductive medicine. Things began to change upon the initial reports of successful surgical sperm retrieval. In vitro fertilization which involves the fertilization of eggs and sperm outside the body in a laboratory setting. Once, an embryo or embryos form, they are placed in the uterus. There are 5 basic steps in the IVF and embryo transfer process which include the collection of ova, collection of sperms, monitoring as well as stimulating the development of healthy ovum/ova in the ovaries, fusion of nurtured ova and desired sperms in the laboratory by providing the appropriate environment for fertilization and early embryo growth, and finally followed by transferring the embryos into the uterus.[47]

First, fertility medications are prescribed to control the ova maturation and to increase the chance of collecting multiple ova during the menstrual cycle referred to as ovulation induction. Multiple ova are desired because some will not develop or fertilize after retrieval. Ovum development is monitored by ultrasound as well as the examination of urine or blood test samples to measure hormone levels. Ova are obtained via laparoscopic or transvaginal ultrasound-guided aspiration.[48]

Second follicular aspiration is achieved by retrieving ova through a minor surgical procedure using ultrasound imaging to guide a hollow needle through the pelvic cavity to remove ova from the ovaries.

Third, sperm are obtained via ejaculation and the sperm and ova are placed in incubators located in the laboratory which enabled fertilization. In cases with lower levels of fertilization, intracytoplasmic sperm injection (ICSI) may be introduced. The ova are monitored to confirm that fertilization and cell division are taking place. They are considered embryos after successful fertilization. The embryos are usually transferred into the woman's uterus from 1 to 6 days later, but in most cases the transfer occurs between 2 to 3 days following egg retrieval.[49] At this stage, the fertilized egg has developed into a 2- to 4-cell embryo. The transfer process involves a speculum which is inserted into the vagina to expose the cervix. A predetermined number of embryos are suspended in fluid and gently placed through a catheter into the womb. Intracytoplasmic sperm injection combined with options for micromanipulation revolutionized treatment of infertile couples. The procedure involves injecting a single sperm, even a nonmotile one, directly into the ooplasm.[49] These investigators developed a novel static method known as time lapse imaging (TLI) to study artificially fertilized embryo without removing them from the incubator to minimize the exposure of embryo to environmental changes, such as temperature, pH, or humidity. They further suggested that TLI enables continuous monitoring of early embryonic development via acquisition of images every 5 to 20 minutes thus minimizing multiple pregnancies, which is a major problem encounter during traditional morphological evaluation.

However, even when men have evidence of spermatogenic dysfunction (detected by semen analysis, DNA fragmentation assay, or sperm functional assays), there is no opportunity to identify those individual sperm with greatest reproductive competence in a given specimen. At present, the only assays that attempt to isolate the most reproductively competent sperm after standard washing techniques have limited clinical data to support their use. Intracytoplasmic morphologically selected sperm injection (IMSI) allows morphology evaluation at high powered magnification. The pregnancy rate were correlated with higher oocyte yields and higher numbers of embryos transferred in the IMSI group compared with the ICSI group, suggesting that the IMSI groups included patients with a better prognosis.[50] Failing injection of single spermatozoa, extraction of testicular sperm can be used.

Karyotyping is important in men with a sperm count <5 million/mL. These individuals show a much higher rate of autosomal abnormalities than fertile populations (around 4%), while the highest frequency is found in azoospermia men (mostly Klinefelter syndrome). Klinefelter syndrome (47, XXY including variants [48, XXXY], and XX males [SRY+ and SRY−]) is the most common of the sex chromosomal aneuploidies. The benefits of knowing if there is a chromosomal abnormality are in the planning for therapy and in the future follow-up of the patient. As such, karyotype analysis should be performed prior to either use of ejaculated sperm in conjunction with ICSI or prior to operative testis sperm extraction.[51]

The other technique for sperm selection is hyaluronic acid (HA) binding assay. This is based on membrane alterations occurring during normal spermiogenesis resulting of occurrence of HA binding sites. Hyaluronic acid bound sperm showed lower rates of aneuploidy and apoptosis thus increasing ICSI improvements in implantation rates among embryos derived from oocytes injected with HA bound sperm.[52]

Herbal Medicine

In traditional medicine, various herbal plants are used to treat male infertility. *Cardiospermum helicacabum* or "Welpenala"

is one such example.[53] The aqueous extract improved sperm count, sperm motility, number of implantations, and viable embryos at 100 and 200 mg/kg dose levels. Similarly, Chinese herbal medicine such as Ginseng roots (*Panax quinquefolius*) improves overall fertility; Tribulus fruit (*Tribulus terrestris*) improves sperm count, morphology, and motility; Maca root (*Lepidium meyenii*) improves hormonal balance.[54]

Future Focus

This laboratory technique is used when a man has a low sperm count and involves selecting his best quality sperms and injecting one into each mature egg produced by the female partner.

In the modern world, about 2% to 4% of births in developed countries are as a result of assisted reproductive technology (ART).[55] Currently, the techniques involve selecting good quality sperm into the mature egg. In the future, the use of the stem cell population should be investigated. Field of omics and ART should be integrated and should be studied in depth to improve future diagnostic tools and to enhance therapeutic approaches.[56]

The selfish spermatogonial stem cell theory suggests that the volume of de novo mutations vary among sperm. Epigenetic modifications also vary within a single ejaculate. Therefore, even sub fertile men who produce a greater proportion of compromised sperm likely also produce many reproductively competent sperm. If the latter could be isolated and used clinically, it is very likely that normal embryogenesis and improved pregnancy outcomes can be achieved. Raman spectroscopy with microfluidics devices could permit temporary trapping of individual sperm to allow for molecular analysis and then forcing of favorable sperm into different channels for clinical use.[52]

Preimplantation genetic screening (PGS) for aneuploidy can be used to select embryos with the highest chance of implantation, to facilitate elective single embryo transfer, and to reduce the risk of chromosomal abnormalities in the offspring. The main problem encountered during ART is that of embryos with abnormal chromosomal numbers or "embryo mosaicism." It is thought that embryo mosaicism arises during the completion of the second meiotic division of the egg, which takes place subsequent to fertilization. Factors such as cell cycle dysregulation, gain or losses of entire chromosomal regions, and amplification of centrosomes are believed to be the main causative mechanisms of embryo mosaicism. An accurate method of detecting mosaic embryos is important as transfer of these embryos would carry a high risk of miscarriage. On the other hand, discarding aneuploid and mosaic embryos using PGS techniques could potentially result in the loss of embryos that have the potential to develop into a normal baby as some mosaic embryos can give rise to normal babies. This area of controversy should be solved in the future.[57]

One high priority area for research was to gain a better understanding of the production, formation, and workings of a human spermatozoon. There is an urgent requirement to understand these cellular, molecular biochemical, and genetic mechanism(s) in order to formulate appropriate diagnostic assays, develop rational therapy for the male, and understand how external factors, such as the environment, negatively or positively influence these processes.[51] An additional high priority area for research is to examine the long-term health outcomes of the children born from men with compromised fertility whatever the nature of the compromising event(s) such as genetics, environmental, iatrogenic, and/or occupational.[58]

It is important to identify the most effective educational initiatives that will improve our understanding of male infertility. Finally, it is now high time for the World Health Organization to produce a 6th version of the semen assessment manual. The evidence based for the current (5th version) manual is at least 10 years out of date and a lot has changed since then. It is vital to encompass educating future learners by integrating to reproductive health in school education system.[59] In addition to the prevailing evaluation criteria of infertile males, country-specific or region-specific counselling and treatment modalities should be established.[60]

Conclusion

To combat infertility, it is essential to optimize lifestyle factors in order to maximize fertility. Sedentary lifestyle, obesity, smoking, heat exposure, stress, poor nutrition, and harmful environmental toxicants may all adversely affect sperm count and quality. Hence, it is important to be aware of harmful chemicals, to be more active, and finally, to live a healthy lifestyle. Simply put, just simple lifestyle changes can improve male fertility. However, in other cases, if natural conception is impossible, assisted reproduction techniques can overcome the problem and advanced techniques such as ICSI treatment can be used. Identifying risk factors to improve the management of human wellness and health throughout standardized analysis, which correlates the accumulation of biotoxins in the seminal fluid with semen quality can be considered in the agenda of public prevention policies

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