Some of the Factors Involved in Male Infertility: A Prospective Review

Abstract: Infertility is defined as the inability of couples to have a baby after one year of regular unprotected intercourse, affecting 10 to 15% of couples. According to the latest WHO statistics, approximately 50–80 million people worldwide suffer from infertility, and male factors are responsible for approximately 20–30% of all infertility cases. The diagnosis of infertility in men is mainly based on semen analysis. The main parameters of semen include: concentration, appearance and motility of sperm. Causes of infertility in men include a variety of things including hormonal disorders, physical problems, lifestyle problems, psychological issues, sex problems, chromosomal abnormalities and single-gene defects. Despite numerous efforts by researchers to identify the underlying causes of male infertility, about 70% of cases remain unknown. These statistics show a lack of understanding of the mechanisms involved in male infertility. This article focuses on the histology of testicular tissue samples, the male reproductive structure, factors affecting male infertility, strategies available to find genes involved in infertility, existing therapeutic methods for male infertility, and sperm recovery in infertile men.

Keywords: male infertility, spermatogenesis, azoospermia, non-obstructive azoospermia

Introduction

Infertility is defined as the inability of couples to have a baby after one year of regular unprotected intercourse, affecting 10–15 percent of couples. According to the latest WHO statistics, about 50–80 million people worldwide suffer from infertility. Large-scale studies have shown that about half of all cases of infertility occur due to female factors, 20 to 30 percent male factors, and 20 to 30 percent due to common causes of both gender. Recent meta-analysis studies by researchers show that male factors are present in 20–70 percent of infertility cases. These findings are significantly broader than previously reported. However, the wide range of male infertility in meta-analysis studies may not reflect the prevalence of this complication in all parts of the world because of reasons such as the lack of rigorous statistical methods that include bias, heterogeneity in data collection, and cultural constraints. Given the significant contribution of male factors to infertility in couples, as well as high levels of unknown factors in male infertility, a lack of understanding of the underlying mechanisms seems to be one of the most important challenges facing this problem. In this article, we have reviewed the histological studies of testicular tissue specimens, male reproductive structure, factors influencing male infertility, strategies to find genes involved in infertility, available therapeutic methods for male infertility, sperm recovery methods in infertile men, and assisting reproductive method (Figure 1).
Male’s Reproductive Organ

In order to better understand the issues and problems associated with infertility, we first discuss some of the key elements involved in male fertility. Human reproductive organs include the primary and secondary organs. Primary reproductive organs include the gonads (responsible for gamete and hormone production), while the secondary organs include the ducts and glands, which play a role in the growth, maturation and transmission of gametes.10,11 The testicles are the primary male reproductive organs enclosed by the tunica albuginea capsule in the testicle sack. Two morphologically and functionally separated parts are in the testis. Tubular components include seminiferous tubules and intercellular portions between seminiferous tubules. The intertubular portions of the seminiferous tubules are involved in providing blood and immune responses.12–14 Leydig cells are one of the most important cells in testis that are the source of testicular testosterone and insulin-like factor 3. In addition to Leydig cells, intercellular components include immune cells, lymphatic and blood vessels, nerves, connective tissue, and fibroblasts.15–18 The seminiferous tubules are functional units in the testis, accounting for 60–80 percent of testicular volume.19–21 These tubes are surround by epithelial tissue and include two types of cells: Sertoli cells and spermatogenic cells. The function of Sertoli cells is to nourish and develop sperm through the stages of spermatogenesis and their mechanical support.22–24 These cells produce two types of inhibin and activin hormone that have positive and negative feedback to FSH.25–27 In addition, Sertoli cells control the stages of sperm release into the lumen, phagocytosis of the degraded germ cells and additional cytoplasm resulting from sperm release. In adulthood, Sertoli cells are meiotically inactive.28–30 Sertoli cell division terminates concurrently with the first meiotic division of the germ cells, giving rise to tight junctions between these cells, known as the Blood-Testis Barrier (BTB) (Figure 2).31,32 The epithelium of seminiferous tubules is divided into two (functionally different) regions by BTB. Two important functions for BTB are: (a) the physical separation of the germ cells that protect them against the immune system; (b) providing an environment for meiosis and sperm development.33–35

Spermatogenesis

Spermatogenesis is one of the most crucial stages in male fertility.36–39 The slightest deviation from the natural course
of spermatogenesis can lead to infertility in men. The term spermatogenesis is a description of the development of male gametes in the seminiferous epithelial tissue from diploid spermatogonia that results in the release of differentiated haploid germ cells into the seminiferous tubules. Each cycle of spermatogenesis in humans requires 16 days and almost 4.6 cycles for development and differentiation of spermatogenic cells into adult sperm, which takes approximately 74 days in humans. The regulation of spermatogenesis occurs in two main stages: a) hormonal and endocrine b) paracrine and autocrine. Many studies have shown that testosterone and FSH are required to successfully complete spermatogenesis. The spermatogenesis process divided into four general phases: 1) mitotic proliferation and spermatogonial differentiation into pre-leptotene spermatocytes (spermatogoniogenesis); 2) Meiotic division of spermatocytes that leads to spermatids (meiosis); 3) Conversion of round spermatids into adult spermatids (spermiogenesis); 4) Release of elongated spermatids into the lumen (spermato genesis) (Figure 3). Considering the importance of spermatogenesis and since the disorder at any of its stages can have irreversible consequences, below are some of the most important features of each stage.

**Spermatogoniogenesis**

The germ cell lines originate from the primary germ cell (PGC). In humans, PGCs develop between endoderm cells at the end of the third week of development, and by the fifth week they migrate to the genital tract, where the presence of the Y chromosome results in the proliferation and transformation of the genital tract into primary male sexual organs. PGCs are commonly called gonocytes during the first trimester of mitosis, then stop in the G3 phase of the cell cycle and remain silent until birth (i.e., when they become spermatogonia). Spermatogonia remain silent until puberty. Spermatogenesis begins with the mitotic proliferation of spermatogonia after birth.
Spermatogenesis during puberty is probably initiated by the production of bone morphogenetic protein 8B (BMP8B). Mice with lack of Bmp8b do not initiate spermatogenesis at puberty and consequently are infertile. Two distinct fates await reproductive cells: (a) self-renewal by replication; (b) becomes spermatozoa. Apoptosis in spermatogonia rarely occurs in the human seminiferous epithelial tissue, but the rate of apoptosis is increased in patients with impaired spermatogenesis, especially in spermatocytes and spermatids.

**Meiotic Division**

Meiosis is the distinction between sexual reproduction and non-sexual reproduction. Meiosis eventually results in the production of haploid gametes from diploid cells. During mammalian meiosis, nuclear division is done twice in a cycle of DNA replication. Each meiosis division is generally divided into two stages Meiosis I and Meiosis II.

In meiosis I, also called subtractive division, the microtubules are attached to sister chromatids via the kinetochore and transported to opposite poles. This transition leads to a decrease in the number of chromosomes from diploid to haploid. Meiosis II is an equal division, in which the microtubules attach to the kinetochore of centromere and separate the sister chromatids, resulting in the formation of four daughter haploid cells. Meiosis begins with the production of two pre-leptotene spermatocytes from spermatogonia. In meiosis I, primary spermatocytes become two secondary spermatocytes, and these cells then form spermatids in meiosis II. The result of meiosis is four different (genetically) cell types.

**Spermogenesis**

Spermogenesis is a process that transforms the meiosis II final product (i.e., spherical spermatids) without splitting into specialized elongated spermatids. This process requires the development of the cytoplasm and nucleus regeneration, which can comprise four distinct phases: the Golgi phase, the capping phase, the acrosomal phase, and the maturation phase.

**Spermatogenesis**

Sperm production is the final stage of spermatogenesis, which mature spermatids are released from the somatic supporting Sertoli cells into the lumen of the seminiferous tubules. At this stage, the cells are known as spermatozoa and continue their journey to epididymis. Seminiferous spermatozoa have low motility and fertility. Spermatozoa passage through the epididymal duct is crucial for final maturation and ability to move. A small amount of cytoplasmic content, cytoplasmic droplets remain in the neck region and the middle segment of the spermatozoa, which facilitates the achievement of epididymis. During the transition from epididymis, which takes approximately two weeks, the cytoplasmic droplets move and exit during the spermatozoa tail, which is associated with increased spermatozoa movement. This event is associated with an increase in the movement of spermatozoa.

**Main Causes of Infertility**

As mentioned, infertility can have a feminine or masculine origin, with the male factor only present in one third of cases. The diagnosis of infertility in men is mainly based on semen analysis. Unusual parameters of semen include: sperm concentration, appearance and motility. There are seven main cases of semen-related abnormalities. Infertility in men can be due to a variety of causes, however, in almost 40% of infertile men there is no clear etiology. There are various reasons for male infertility, the most important of which are: Hormonal deficits, physical causes, sexually transmitted problems, environment and lifestyle, and genetic factors.

**Hormonal Defects**

The male reproductive hormone axis is known as the hypothalamic-pituitary-gonadal axis. It consists of 3 major components: the hypothalamic, pituitary and testicular glands (Figure 4). This axis works very regularly to provide the right concentration of hormones for male sexual development and function. Any abnormality in the system can lead to infertility. If the brain is unable to produce gonadotropic releasing hormone (GnRH), this disorder results in a lack of testosterone and stopping sperm production. Lack of GnRH causes a group of disorders known as hypogonadotropic hypogonadism. One of them is known as Kallmann syndrome, which is associated with a change in sense of smell and immaturity. Treatment options for gonadotropin-releasing hormone deficiency include: Use of sex steroids, gonadotropins and injection of gonadotropin releasing hormone. Testosterone injections are mainly used to improve testicular growth, normalize testosterone concentration, and stimulate the development of secondary sexual traits. Similarly, the pituitary’s inability to produce sufficient amounts of luteinizing hormone and follicular stimulating hormone results in a failure to stimulate the testes and to produce testosterone and
sperm. Patients with pituitary deficiency require long-term hormonal therapy, which can lead to complications such as diabetes mellitus, heart disease and bone defects. Conversely, elevated concentrations of LH and FSH are associated with low concentrations of testosterone, leading to defects in spermatogenesis. Therefore, using high doses of testosterone and estrogen can be a viable treatment option because it suppresses the production of LH and FSH. Increased prolactin can also lead to reduced sperm production, libido and impotence. Hyperprolactemia leads to infertility in 11% of people with oligospermia. In many cases, a dopamine agonist can be a good treatment.

Physical Reasons
Physical problems can disrupt sperm production and blockage of the ejaculatory pathway. Enlargement of the sperm vessels known as varicocele is one of the most common male infertility problems affecting about 40% of men. Testicular torsion within the testicle sac can cause testicular damage due to pressure on the sperm vessels and impaired testicular circulation. Chronic and acute genital tract infections can also be common causes of infertility in men. Mumps viral infection can lead to testicular atrophy and infertility. Sexually transmitted diseases such as Gonorrhea and Chlamydia can also lead to infertility in men due to obstruction in the epididymis. In some cases, semen is ejaculated in the bladder, known as recurrent ejaculation, and accounts for about 2% of infertility cases that can be caused by anatomical problems of the bladder sphincter.

Sexual Problems
Many sexual problems are both physical and psychological. Erectile dysfunction, known as impotence, early ejaculation and inability to ejaculate are examples of intercourse problems.
Environment and Lifestyle
Men exposed to hazardous substances in their workplace, including solvents, insecticides, adhesives, silicones and radiation, exposure to these and similar substances can lead to infertility. Exposure to radiation can lead to reduced sperm production, and exposure to high doses can lead to complete infertility. Overuse of the sun bath can also lead to a temporary decrease in sperm count. Occupations that require prolonged sitting (such as driving) or being exposed to high temperatures (such as bakeries) can have negative effects on fertility. Concerning alcohol consumption and smoking, there is no definite agreement regarding their effect on sperm parameters and fertility outcomes. However, progressive degradation in sperm quality may be associated with cigarette smoking and alcohol consumption. Poor nutrition can also play an important role in male infertility. There has been a recent report of a decrease in sperm concentration in men with an increase in saturated fat intake. Repeated use of drugs such as cocaine and cannabinoids is associated with a significant decrease in sperm concentration, and urinary testosterone in men. In addition, studies have also shown that air pollution in men reduces sperm motility, and the way to deal with and prevent this problem is to continually use antioxidants and vitamin C-containing substances. Moreover the presence of pollutants and sulfur dioxide in the air changes the natural shape of sperm and also has a detrimental effect on sperm motility.

Genetic Factors
Genetic factors are detected in 15% of male infertility cases and can be classified into two groups: chromosomal abnormalities and single-gene mutations. Any lack or acquisition of unusual rearrangements in genetic material at the chromosomal level is known as chromosomal abnormalities and is one of the major genetic causes involved in male infertility. About 14% of men with azoospermia and 2% of men with oligospermia have chromosomal abnormalities, which is much higher than the general population (about 0.6%). Some chromosomal abnormalities are inherited and some are acquired. The most common genetic cause of azoospermia in the aneuploid sex chromosome is Klinefelter syndrome, which accounts for about 14% of male infertility cases. 47,XYY, chromosomal defects can cause spermatogenesis malfunction due to increased FSH and Y chromosome disomy. Noonan syndrome in men, such as Turner syndrome in women, which is XO/XY mosaic, can lead to cryptochidism and spermatogenesis deficiency due to increased FSH. Translocations occur in 3% of patients with severe oligozoospermia, the most important is Robertsonian and bilateral translocation. Inversion is called chromosomal translocation, in which a fragment of the chromosome is broken and rearrangement within itself. Autosomal inversions are eight times more frequent in infertile men, although these rearrangements are balanced, in some cases leading to severe oligoasthenoteratozoospermia or azoospermia. The role of the Y chromosome was identified by Zofardi and colleagues by karyotype analysis of deletions in the long arm of the Y chromosome in six infertile men, they termed the deletion region as azoospermic factor (AZF). This region contains three zones AZFa, AZFb, and AZFc. Micro-deletions occur following the recombination of similar fragments in palindromic sequences. Y chromosome micro-deletions are present in 10% of infertile men, whereas in oligozoospermic males, the prevalence is 7%. The most common microdeletion occurs in the AZFc region, accounting for 80% of cases. Deletions that encompass the entire AZFa region result in the Sertoli cell phenotype. Intra-AZFb deletion usually results in azoospermia. Deletion in the AZFc region can lead to a wide range of infertility phenotypes including azoospermia, Sertoli cell syndrome and oligozoospermia. Some gene mutations with pathological syndromes can be associated with infertility, such as congenital bilateral absence of the vas deferens (CBAVD), which cause obstructive azoospermia in 80 to 90% of cases. This defect is caused by a mutation in the Cystic fibrosis transmembrane regulator (CFTR). Primary ciliary defects are an autosomal recessive heterogeneous defect caused by a lack of normal eyelash function and present in half of men with asthenospermia. Little has been known so far about non-syndromic infertility.

Epigenetic Factor
Acetylation and methylation are two effective factors in epigenetic modifications that cause different expression of genes. Epigenetic factors act a critical role in male infertility, and numerous studies have been devoted to it. During spermatogenesis, germ cells face major epigenetic reprogramming that includes the organization of sex-specific designs in the sperm, which substitution of histone to protamine is one of them. Numerous experiments have revealed altered epigenetic function in sperm from men with oligozoospermia and oligoasthenoteratozoospermia. Besides, many studies have been reported that...
hypermethylation in several genes, lead to deficiency in semen parameters or male infertility.\textsuperscript{151–153}

**Strategies for Finding Genes Involved in Infertility**

There are two general approaches to infertility studies for finding genes involved in infertility: the candidate gene approach and the whole genome approach. A) The candidate gene approach; Identification of genes that lead to impaired fertility in model animals (mostly mice), and assuming that their function is maintained during evolution, these genes are selected and their roles and effects in human infertility are studied. It is important to note that in this method, the function and expression of candidate genes in model animals and their effect on infertility have already been proven, and given the foregoing, it is possible to predict the gene involved in human infertility. B) Whole-genome approach; technological advances in whole-genome studies such as single-nucleotide polymorphism (SNP) microarray, high-throughput sequencing technologies such as exome or whole-genome sequencing, and their use for finding effective genes in infertility has been considered.\textsuperscript{154,155} In single nucleotide polymorphisms or SNPs, the difference in one nucleotide causes different phenotypes. SNPs are classified into common and non-common groups based on allele frequency. Sequencing technologies enable researchers to perform high-throughput sequencing, which allows millions of pieces of DNA to be sequenced. Exome sequencing is another field that has revolutionized the study of a variety of disorders, including infertility. Exom covers about 1% of the entire human genome but accounts for about 85% of the pathogenic mutations.\textsuperscript{156} Exome sequencing allows us to identify mutations in the protein coding region. On the other hand, whole-genome sequencing can identify potentially susceptible mutations throughout the genome. Genome-wide association studies (GWAS) have been able to identify different polymorphisms related to defects in spermatogenesis. Until now, however, genetic risk factors identified with this technique have shown poor association.

After introducing some of the influencing factors in infertility and exploring its identification methods, the challenge that remains to be resolved is how to use the information obtained to treat diagnosed infertility. Following some of the most up-to-date and important strategies for treating infertility with a specific cause, are mentioned.

**Fertility Assistance Techniques**

Although different definitions have been proposed, assisted reproductive techniques refer to a range of methods generally used to treat infertility problems in humans and help infertile couples to have a healthy child. There are three main stages of progressive intervention in this area. A) Stimulation of ovulation during intercourse. B) Stimulation of ovulation and injection of sperm into the female reproductive tract. C) Artificial fertilization in which the egg and sperm are fertilized outside the body and the resulting embryo is transferred into the uterus. Each of these steps is discussed below.

**Auxiliary Fertilization**

This process involves controlled ovarian stimulation to increase the maturation of several oocytes, egg harvest through follicle aspiration, sperm recovery, laboratory inoculation, and embryo transfer and culture. Although assistant fertilization can be performed in the normal sex cycle, the most common protocol involves the daily injection of recombinant human FSH to stimulate follicle growth to obtain the most oocytes.\textsuperscript{157,158} The follicles are monitored by serum estradiol and uterine ultrasound. Once the follicles have reached the appropriate size, human chorionic gonadotropin (HCG) is injected to stimulate follicle maturation and is collected 32 to 36 hrs after injection. Although frozen sperm can also be used according to WHO guidelines; Generally, on the day that eggs are collected, semen is also collected by masturbation after a period of 2 to 7 days of abstinence for artificial insemination. After egg stimulation and sperm recovery, two methods of fertilization are used: IVF and ICSI.\textsuperscript{159,160}

**Sperm Recovery in Infertile Men**

For infertile men, sperm must be recovered directly from the testicles or epididymis. Obstructive azoospermia (OA) and non-obstructive azoospermia\textsuperscript{51} are two major categories of azoospermia.\textsuperscript{161} Obstructive azoospermia is the result of physical obstruction of the male genital tract, which may be due to or acquired factors (i.e., infection, vasectomy or physical injury to the genital tract), congenital absence of the vas deferens (congestion of the vas deferens, which accounts for about 60% of men with azoospermia), epididymal obstruction.\textsuperscript{162} On the other hand, NOA is due to the lack of testicular sperm production in the ejaculate.\textsuperscript{163,164} The best way to treat NOAs is to extract sperm from the testis (TESE) and done.
intracytoplasmic sperm injection (ICSI). However, in half of the cases of azoospernia, sperm cannot be found as a result of TESE.\textsuperscript{165} Unfortunately, serum hormone levels such as FSH and inhibin B and noninvasive assessments such as testicular volume cannot predict sperm recovery and to date only testicular histopathology can be used as a predictor of successful sperm recovery rate (SSR).\textsuperscript{166} In the conventional TESE method, spermatozoa are extracted from testicular biopsies by local or general anesthesia.\textsuperscript{167} On the other hand, sperm extraction is much safer and more successful by micro-TESE.\textsuperscript{168}

The purpose of the micro-TESE is to identify the nuclear regions of testicular sperm production based on the size and appearance seminiferous tubules with the aid of a microscope, in which spermatozoa can be recovered from open seminiferous tubules, the whole process being visible under the microscope. Micro-TESE is a better alternative to TESE because of the increased chance of sperm recovery and reduced testicular damage due to the smaller size of the harvested tissue. In general, testicular tissue resection for histopathologic evaluation can potentially eliminate sites that still produce sperm, despite abnormalities.\textsuperscript{169,170} Testis biopsy before sperm recovery is generally not recommended. Testicular biopsy is usually performed on the day of egg retrieval. Biopsy specimens are examined for the presence of sperm. A small sample is taken from an accessible area and evaluated for histopathology.\textsuperscript{170–172} Due to the uncertainty of sperm retrieval and failure of sperm retrieval, egg retrieval will be unnecessary. This can cause emotional, economic, and physical stress for couples, so sperm retrieval requires the use of predictive factors, and this will not be possible unless you have in-depth knowledge of all the steps that can lead to Infertility in men.

**Discussion**

Today, around 10–15\% of couples around the world are experiencing infertility (60–80 million people). In half of cases, male infertility is the cause. Disruption of spermatogenesis is a major cause of infertility, and genetic abnormalities affecting spermatogenesis can be the cause of many unknown male infertility cases. Therefore, identifying and presenting prognostic biomarkers as well as finding non-invasive therapeutic techniques seem necessary. In hormonal investigations, the micro-TESE technique can increase the biomarker value of FSH to predict sperm recovery; because the results of hormonal studies show the whole process of spermatogenesis in all areas of the testis. Given that the testicular tissue is heterogeneous, the micro-TESE technique can increase the likelihood of sperm retrieval despite inadequate levels of the FSH hormone, due to sampling small areas of testicular tissue.

**Conclusion**

Given that male infertility in many cases remains unknown. Therefore, it is necessary to introduce new key factors and diagnostic and noninvasive biomarkers. Over the past few years, the identification and evaluation of small noncoding RNAs in many diseases, including infertility, has helped greatly in understanding the underlying mechanisms of disease. But this alone is not enough, and through increased insight into the complex stages and processes of pregnancy in humans, more key elements must be identified so that infertile couples can enjoy the chance of a natural pregnancy in addition to reducing costs and problems. With the advances in technology and the introduction of new methods and approaches, it is hoped that many of the causes of male infertility will soon be identified and treated.

**Abbreviations**

BTB, Blood-Testis Barrier; PGC, primary germ cell; GnRH, gonadotropin releasing hormone; AZF, azoospermic factor; CBAVD, congenital bilateral absence of the vas deferens; CFTR, cystic fibrosis transmembrane regulator; SNP, single-nucleotide polymorphism; GWAS, genome-wide association studies; HCG, human chorionic gonadotropin; OA, obstructive azoospermia; NOA, non-obstructive azoospermia; TESE, testicular sperm extraction; ICSI, intracytoplasmic sperm injection; SSR, sperm recovery rate.

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**Author Contributions**

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

**Disclosure**

All authors declare that they have no conflicts of interest.

**References**

1. ESHRE Capri Workshop Group, Albertini DF, Anderson R, Bhattacharya S, et al. A prognosis-based approach to infertility: understanding the role of time. *Hum Reprod*. 2017;32(8):1556–1559. doi:10.1093/humrep/dex214
2. Turchi P. Prevalence, definition, and classification of infertility. In: Cavallini G, Beretta G, editors. Clinical Management of Male Infertility. Springer; 2015:5–11.

3. Vander Boght M, Wynn C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018;62(2–3):10. doi:10.1016/j.clinbiochem.2018.03.012

4. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The international glossary on infertility and fertility care. 2017. Hum Reprod. 2017;32(9):1786–1801. doi:10.1093/humrep/dex234

5. Bricae I, Costache A, Puncareva Y, et al. Fallopian tubes-literature review of anatomy and etiology in female infertility. J Med Life. 2015;8(2):129.

6. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci. 2015;8(4):191. doi:10.4103/0974-1208.170370

7. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13(1):37. doi:10.1186/s12978-015-0032-1

8. Masoumi SZ, Parsa P, Darvish N, Mokhtari S, Yavangi M, Roshanaei G. An epidemiologic survey on the causes of infertility in patients referred to infertility center in Fatemieh Hospital in Hamadan. Iran J Reprod Med. 2015;13(8):513.

9. Qi X, Wang K, Zhou G, Xu Z, Yu J, Zhang W. The role of testicular artery in laparoscopic varicocelectomy: a systematic review and meta-analysis. Int Urol Nephrol. 2016;48(5):955–965. doi:10.1007/s11255-016-1254-7

10. Ampatzidis G, Georgakopoulou D, Kapsi G. Citloris, the unknown: what do postgraduate students of educational sciences know about reproductive physiology and anatomy? J Biol Educ. 2019;1–10. doi:10.1080/00219266.2019.1679658

11. Carson SA, Buster JE, Cesario M, Woodard SP. Recovery and isolation of Sertoli cells and peritubular cells from rat testes. J Hum Reprod Sci. 2015;8(2):103. doi:10.4103/0974-1208.158618

12. Orman D, Vardi N, Ates B, Taslidere E, Elke H. Aminoguanidine mitigates apoptosis, testicular seminiferous tubules damage, and oxidative stress in streptozotocin-induced diabetic rats. Tissue Cell. 2015;47(3):284–290. doi:10.1016/j.tice.2015.03.006

13. Mehrabani D, Hassanshahi MA, Tamadon A, et al. Adipose tissue-derived mesenchymal stem cells repair germinal cells of seminiferous tubules of busulfan-induced azoospermic rats. J Hum Reprod Sci. 2015;8(2):103. doi:10.4103/0974-1208.158618

14. Pitia AM, Minagawa I, Uera N, et al. Expression of insulin-like factor-1 in Sertoli cells. Cell Tissue Res. 2015;362(2):407–420. doi:10.1007/s00441-015-2206-8

15. Ye L, Li X, Li L, Chen H, Ge R-S. Insights into the development of the adult Leydig cell lineage from stem Leydig cells. Front Physiol. 2017;8:430. doi:10.3389/fphys.2017.00430

16. Bhushan S, Aslani F, Zhang Z, Sebastian T, Elssasser H-P, Klug J. Isolation of Sertoli cells and peritubular cells from rat testes. J Vis Exp. 2016;108:e53389.

17. Shah S, Saini N, Ashraf S, et al. Comparative expression analysis of gametogenesis-associated genes in foetal and adult bulbarine (Bubalus bubalis) ovaries and testes. Reprod Domest Anim. 2015;50(3):365–377. doi:10.1111/rdia.2015.50.issue-3

18. Duan P, Hu C, Quan C, et al. 4-Nonylphenol induces apoptosis, autophagy and necrosis in Sertoli cells: involvement of ROS-mediated AMPK/AKT-mTOR and JNK pathways. Toxicology. 2016;341–343:28–40. doi:10.1016/j.tox.2016.01.004

19. Yao C, Sun M, Yuan Q, et al. MiRNA-133b promotes the proliferation of human Sertoli cells through targeting GLI3. Oncotarget. 2016;7(2201). doi:10.18632/oncotarget.v7i13

20. Zhang L, Chen M, Wen Q, et al. Reprogramming of Sertoli cells to fetal-like Leydig cells by Wt1 ablation. Proc Natl Acad Sci. 2016;113(12):4003–4008. doi:10.1073/pnas.1422371112

21. Cortes D, Clasen-Linde E, Hutson JM, Li R, Thorup J. The Sertoli cell hormones inhibit-B and anti Müllerian hormone have different patterns of secretion in prepubertal cryptorchid boys. J Pediatr Surg. 2016;51(3):475–480. doi:10.1016/j.jpedsurg.2015.08.059

22. Dimitriadis F, Tsiampali C, Chaliasos N, Tsounapi P, Takenaka A, Sofikitis N. The Sertoli cell as the orchestra conductor of spermatogenesis: seminiferous cells dance to the tune of testosterone. Hormones. 2015;14(4):479–503. doi:10.14310/ horm.2002.1633

23. Loveland KL, Hedgey MP. Activins and inhibins in Sertoli cell biology: implications for testsis development and function. In: Griswold MD, editor. Sertoli Cell Biology. Elsevier; 2015:201–232.

24. Chiaichanathong S, Taya K, Watanabe G, et al. Immunohistochemical localization of inhibin/activin subunits in adult Asian elephant (Elephas maximus) testes. J Vet Med Sci. 2018;80(3):549–552. doi:10.1292/jvms.17-0753

25. Li Y, Fortin J, Ongaro L, et al. Betaglycan (TGFBR3) functions as an inhibitor of Sertoli cell proliferation of human Sertoli cells through targeting GLI3. Front Mol Biosci. 2015;2:104. doi:10.3389/fmolb.2015.00120

26. Loveland KL, Hedgey MP. Activins and inhibins in Sertoli cell biology: implications for testsis development and function. In: Griswold MD, editor. Sertoli Cell Biology. Elsevier; 2015:201–232.

27. Winters S, Moore J Jr, Clark B. Leydig cell insufficiency in hypospermatogenesis: a paracrine effect of activin–inhibin signaling? Andrology. 2018;6(2):262–271. doi:10.1111/andro.2018.6.issue-2

28. Gerber J, Heinrich J, Brehm R. Blood-testis barrier and Sertoli cell function: lessons from SCCxs4KO mice. Reproduction (Cambridge, England). 2016;152(2):R15–R27. doi:10.1530/REP-15-0366

29. Li N, Murk DD, Lee WM, Wong CK, Cheng CY. Is toxicant-induced Sertoli cell injury in vitro a useful model to study molecular mechanisms in spermatogenesis?. Semin Cell Dev Biol. 2016;59:141–156.

30. Cao X-N, Shen L-J, Yan C, et al. Urban fine particulate matter exposure causes male reproductive injury through destroying blood-testis barrier (BTB) integrity. Toxicol Lett. 2017;266:1–12. doi:10.1016/j.toxlet.2016.12.004

31. Fan Y, Liu Y, Xue K, et al. Diet-induced obesity in male C57BL/6 mice decreases fertility as a consequence of disrupted blood-testis barrier. PLoS One. 2015;10(4):e0120775. doi:10.1371/journal.pone.0120775

32. Zhang J, Li Z, Qie M, Zheng R, Shetty J, Wang J. Sodium fluoride and sulfur dioxide affected male reproduction by disturbing blood-testis barrier in mice. Food Chem Toxicol. 2016;94:103–111. doi:10.1016/j.fct.2016.05.017

33. Goh WSS, Falcatori I, Tam OH, et al. piRNA-directed cleavage of meiotic transcripts regulates spermatogenesis. Genes Dev. 2015;29(10):1032–1044. doi:10.1101/gad.260455.115

34. Griswold MD. Spermatogenesis: the commitment to meiosis. Physiol Rev. 2016;96(1):1–17. doi:10.1152/physrev.00013.2015
38. Gunes S, Al-Sadaan M, Agarwal A. Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. Reprod Biomed Online. 2015;31(3):309–319. doi:10.1016/j.rbmo.2015.06.010
39. O’Hara L, Smith LB. Androgen receptor roles in spermatogenesis and infertility. Best Pract Res Clin Endocrinol Metabol. 2015;29(4):595–605. doi:10.1016/j.beem.2015.04.006
40. Galdon G, Atala A, Sadri-Ardekani H. In vitro spermatogenesis: how far from clinical application? Curr Urol Rep. 2016;17(7):49. doi:10.1007/s11990-016-0605-3
41. Pieri NCG, Souza A, Mançanares ACF, et al. Immunolocalization of proteins in the spermatogenesis process of canine. Reprod Domest Anim. 2017;52:170–176. doi:10.1111/rda.2017.52.issue-S2
42. Xu H, Shen L, Chen X, et al. mTOR/P70S6K promotes spermatogonia proliferation and spermatogenesis in Sprague Dawley rats. Reprod Biomed Online. 2016;32(2):207–217. doi:10.1016/j.rbmo.2015.11.007
43. Garolla A, Ghezzi M, Cosci I, et al. FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. Endocrine. 2017;56(2):416–425. doi:10.1007/s12020-016-1037-z
44. Yao C, Liu Y, Sun M, et al. MicroRNAs and DNA methylation as epigenetic regulators of mitosis, meiosis and spermatogenesis. Reproduction. 2015;150(1):R25–R34. doi:10.1530/REP-14-0643
45. Donovan PJ, de Miguel MP. Turning germ cells into stem cells. J Cell Sci. 2017;130(5):883–883. doi:10.1242/jcs.199346
46. Hsieh SU, Chen LY, Hoda S, et al. Nuclear transplantation for the restoration of VM mice spermatogenesis. Hum Reprod. 2018;33(11):2709–2719. doi:10.1093/humrep/dex312
47. Looijenga LH. Pathogenesis of testicular germ cell tumors. In: Babakhanzadeh et al., editors. Pathogenesis of male germ cell neoplasia. Skakkebæk NE. Developmental arrest of germ cells in the pathogenesis of germ cell neoplasia. Berlin: Springer; 1998. p. 288.
48. Yoon S-R, Dubeau L, de Young M, Wexler NS, Arnheim N. Turnover of FSH receptors in spermatogonia differentiation. Proc Natl Acad Sci U S A. 2003;100(15):8834–8838. doi:10.1073/pnas.1313190100
49. Sluder G, McCollum D. The mad ways of meiosis. Carlsberg Res Commun. 1996;37:167–188. doi:10.1016/S0303-7207(96)0002-4
50. Rasmussen SW, Holm PB. Human meiosis II. Chromosome pair-disjunction and maternal age in meiosis-II human oocytes. Hum Reprod. 2004;19(1):45–50. doi:10.1093/humrep/deh130
51. Breton B, Billard R. Effects of the temperature and of the photoperiod on trout spermogenesis. Science. 2000;289(5474):254–255. doi:10.1126/science.289.5474.254
52. Breton B, Billard R. Effects of the temperature and of the photoperiod on trout spermogenesis. Science. 2000;289(5474):254–255. doi:10.1126/science.289.5474.254
53. Lamb NE, Freeman SB, Savage-Austin A, et al. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. Nat Genet. 1996;14(4):400. doi:10.1038/ng1296-400
54. Rasmussen SW, Holm PB. Human meiosis II. Chromosome pairing and recombination nodules in human spermatocytes. Carlsberg Res Commun. 1978;43(5):275. doi:10.1007/BF02096106
55. Breton B, Billard R. Effects of the temperature and of the photoperiod on trout spermogenesis. Selezione Veterinaria (Italy); 1979.
56. Sapsford C, Rae CA, Cleland K. Ultrastructural studies on spermatids and sertoli cells during early spermogenesis in the bandicoot Peramelea nasuta geoffroy (Marsupialia). Aust J Zool. 1996;44(5):881–909. doi:10.1071/ZO9670881
57. Thamer IK, Hussein FA, Khorsheed HH. Study the event cycles of spermatogenesis in man: an estimate of its duration. Science. 1963;140(3563):184–186. doi:10.1126/science.140.3563.184
58. Phillips BT, Gassei K, Orwig KE. Spermatogonial stem cell regulation and spermatogenesis. Philos Trans R Soc B. 2010;365(1546):1663–1678. doi:10.1098/rstb.2010.0026
76. Sharpe R. Testosterone and spermatogenesis. J Endocrinol. 1987;113(1):1–2. doi:10.1677/pe.0.113001
77. Eddy EM. Regulation of gene expression during spermatogenesis. Semin Cell Dev Biol. 1998;9(4):451–457.
78. Johnson L. Spermatogenesis and aging in the human. J Androl. 1996;7(6):331–354. doi:10.1002/j.1939-4640.1996.tb00943.x
79. Painter TS. Studies in mammalian spermatogenesis. II. The spermatogenesis of man. J Exp Zool. 1923;37(3):291–336.
doi:10.1002/js/ir.0100X
80. De Krester D, Baker H. Infertility in men: recent advances and continuing controversies. J Clin Endocrinol Metab. 1999;84(10):3443–3450.
81. Thonneau P, Marchand S, Tallec A, et al. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988–1989). Hum Reprod. 1991;6(6):811–816. doi:10.1093/oxfordjournals.humreep.a137433
82. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol. 2018;15(6):369–384. doi:10.1038/s41571-018-0003-3
83. Lee JA, Ramasamy R. Indications for the use of human chorionic gonadotropin for the management of infertility in hypogonadal men. Trans Androl Urol. 2018;7(Suppl 3):S348. doi:10.21037/ta.2018.04.11
84. Lotti F, Maggi M. Sexual dysfunction and male infertility. Nat Rev Urol. 2018;15(5):287. doi:10.1038/nruro.2018.20
85. Winters BR, Walsh TJ. The epidemiology of male infertility. Urol Clin. 2014;41(1):195–204. doi:10.1016/j.ucl.2013.08.006
86. Corradi PF, Corradi RB, Greene LW. Physiology of the hypothalamic-pituitary-gonadal axis in the male. Urol Clin. 2016;43(2):151–162. doi:10.1016/j.ucl.2016.01.001
87. Kuri-Hanninen T, Sinkkilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr. 2014;82(2):73–80. doi:10.1159/000362414
88. Costanzo P, Suarez S, Scaglia H, Zylbersztein C, Litwak L, Winters BR, Walsh TJ. The epidemiology of male infertility. Andrology. 2014;2(1):117–124. doi:10.1111/andr.2013.2.issue-1
89. Tsatsanis C, Dermitzaki E, Avgoustinaki P, Malliaraki N, Mytaras V, Margioris AN. The impact of adipose tissue-derived factors on the hypothalamic-pituitary-gonadal (HPG) axis. Hormones. 2015;14(4):549–562. doi:10.14310/horm.2002
90. Xiong X, Zhong A, Xu H. Effect of cyanothrixin on the hypothalamic–pituitary–gonadal axis in male adult mouse. PLoS One. 2014;9(11):e105685. doi:10.1371/journal.pone.0105685
91. Lucas X. Clinical use of deslorelin (GnRH agonist) in companion animals: a review. Reprod Domest Anim. 2014;49:64–71. doi:10.1111/rdia.2014.49.issue-s4
92. Monaco D, Fatnassi M, Padalino B, et al. Effects of a GnRH releasing hormone (GnRH) de
93. Cejko BI, Babakhanzadeh et al. Gynecol Obstet Hum Reprod Dev Med. 2018;2014:35(1):73–79.
94. Balasubramanian R, Crowley WF Jr. Isolated Gonadotropin-Releasing Hormone (GnRH) deficiency. GeneReviews® [Internet]. University of Washington, Seattle; 2017.
95. Pienkowski C, Tauber M. Gonadotropin-releasing hormone agonist treatment in sexual precocity. In: Bourguignon JP, Parent AS, editors. Puberty from Bench to Clinic. Vol. 29. Karger Publishers; 2016:214–229.
96. Schagen SE, Cohen-Kettenis PT, Delemarre-van de Waal HA, Hannema S. Efficacy and safety of gonadotropin-releasing hormone agonist treatment to suppress puberty in gender dysphoric adolescents. J Sex Med. 2016;13(7):1125–1132. doi:10.1016/j.jsxm.2016.05.004
97. Kiezun M, Smolinska N, Maleszka A, Dobrzyk K, Szeszko K, Kamiński T. Adipopectin expression in the porcine pituitary during the estrous cycle and its effect on LH and FSH secretion. Am J Physiol Endocrinol Metabol. 2014;307(11):E1038–E1046. doi:10.1152/apend.00299.2014
98. Wdowiak A, Raczkiewicz D, Stasiak M, Bojar I. Levels of FSH, LH and testosterone, and sperm DNA fragmentation. Neuroendocrinol Lett. 2014;35(1):73–79.
99. Ekman B, Fitts D, Marelli C, Murray RD, Quinkler M, Zelissen PM. European Adrenal Insufficiency Registry (EU-AIR): a comparative observational study of glucocorticoid replacement therapy. BMC Endocr Disord. 2014;14(1):40. doi:10.1186/1472-6823-14-40
100. Fukuda I, Hizuka N, Muraoka T, Ichihara A. Adult growth hormone deficiency: current concepts. Neurol Med Chir (Tokyo). 2014;54(8):599–605.
101. Marrag I, Haji K, Braham MY, Dhifallah M, Nasr M. Antipsychotics and hyperprolactinemia: prevalence and risk factors. Ann Psychiatry Ment Health. 2015;3(6):1047.
102. Fukuda I, Hizuka N, Muraoka T, Ichihara A. Adult growth hormone deficiency: current concepts. Neurol Med Chir (Tokyo). 2014;54(8):599–605.
103. Sengupta P, Agarwal A, Pogrebetskaya M, Roychoudhury S, Durairajnayagam D, Henkel R. Role of Wiltamia sonnifera (Ashwagandha) in the management of male infertility. Reprod Biomed Online. 2018;36(3):311–326. doi:10.1016/j.rbmo.2016.11.007
104. Sun X-L, Wang J-L, Peng Y-P, et al. Bilateral is superior to unilateral varicocelectomy in infertile males with left clinical and right subclinical varicocele: a prospective randomized controlled study. Int Urol Nephrol. 2018;50(2):205–210. doi:10.1007/s11255-017-1749-x
105. Fallahi S, Rostami A, Shiadch MN, Behniafar H, Paktinat S. An updated literature review on male infertility. Reprod Biomed Online. 2018;36(3):311–326. doi:10.1016/j.rbmo.2016.11.007
106. Davison P, Morris J. Mumps. StatPearls [Internet]. StatPearls Publishing; 2018.
107. Tsoumanis A, Hens N, Kenyon CR. Is screening for chlamydia and gonorrhea in men who have sex with men associated with reduction of the prevalence of these infections? A systematic review of observational studies. Sex Transm Dis. 2018;45(9):615–622. doi:10.1097/OLQ.0000000000000824
108. Omoaloye T, Du Plessis SS. Diabetes mellitus and male infertility. 2018.
109. Kabib A. Mechanisms linking obesity to male infertility. Cent European J Urol. 2015;68(1):79. doi:10.5173/ceju.2015.01.435
110. Otunctemur A, Bozkurt M, Besiroglu H, Polat EC, Ozcan L, Ozbek E. Erectile dysfunction is positively correlated with mean platelet volume and platelet count, but not with eosinophil count in peripheral blood. Urol J. 2015;12(5):2347–2352.
111. Mustafa M, Sharifa AM, Hadi J, Illzam E, Aliya S. Male and female infertility: causes, and management. IOSR-JDMS. 2019;18(9):27–32.
112. Rim K-T. Reproductive toxic chemicals at work and efforts to protect workers’ health: a literature review. Saf Health Work. 2017;8(2):143–150. doi:10.1016/j.shaw.2017.04.003
113. Liu K-S, Pan F, Chen Y-J, Mao X-D. The influence of sperm DNA damage and semen homocysteine on male infertility. Reprod Dev Med. 2017;1(4):228. doi:10.4103/2096-2924.224910
154. Aston K. Genetic susceptibility to male infertility: news from genome-wide association studies. Androl. 2014;2(3):315–321. doi:10.1111/andr.2014.2.issue-3

155. Carrell DT, Aston KJ. The search for SNPs, CNVs, and epigenetic variants associated with the complex disease of male infertility. Syst Biol Reprod Med. 2011;57(1–2):17–26. doi:10.3109/19396368.2010.521615

156. Coffey AJ, Kokocinski F, Calafato MS, et al. The GENCODE exome: sequenced the complete human exome. Eur J Hum Genet. 2011;19(7):827. doi:10.1038/ejhg.2011.28

157. Boxmeer JC, Smit M, Weber RF, et al. Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. J Androl. 2007;28(4):521–527. doi:10.2164/jandrol.106.001982

158. Collins JA. An international survey of the health economics of IVF and ICSI. Hum Reprod Update. 2002;8(3):265–277. doi:10.1093/humupd/8.3.265

159. Haagen E, Tuil W, Hendriks J, Braat D, Kremer J. Current internet use and preferences of IVF and ICSI patients. Hum Reprod. 2003;18(10):2073–2078. doi:10.1093/humrep/deg423

160. Kushnir VA, Frattarelli JL. Aneuploidy in abortuses following IVF and ICSI. J Assist Reprod Genet. 2009;26(2–3):93–97. doi:10.1007/s10815-009-9292-z

161. Friedler S, Raziel A, Strassburger D, Schachter M, Soffer Y, Ron-El R. Factors influencing the outcome of ICSI in patients with obstructive and non-obstructive azoospermia: a comparative study. Hum Reprod. 2002;17(12):3114–3121. doi:10.1093/humrep/17.12.3114

162. Hosnitzer M, Goldstein M, Hardy MP. Review of azoospermia. Spermatogenesis. 2014;4(1):e28218. doi:10.4161/spmg.28218

163. Abdel Raheem A, Garaffa G, Rushwan N, et al. Testicular histopathology as a predictor of a positive sperm retrieval in men with non-obstructive azoospermia. BJU Int. 2013;111(3):492–499. doi:10.1111/bju.12464–410X.2012.11203.x

164. Vernoaeve V, Bondielle M, Tourjane H, Camus M, Van Steirteghem A, Devroey P. Pregnancy outcome and neonatal data of children born after ICSI using testicular sperm in obstructive and non-obstructive azoospermia. Hum Reprod. 2003;18(10):2093–2097. doi:10.1093/humrep/deg403

165. Vloeberghs V, Verheyen G, Haentjens P, Goossens A, Polyzos N, Tournaye H. How successful is TESE-ICSI in couples with non-obstructive azoospermia? Hum Reprod. 2015;30(8):1790–1796. doi:10.1093/humrep/dev139

166. Bohring C, Schroeder-Printzen I, Weidner W, Krause W. Serum levels of inhibin B and follicle-stimulating hormone may predict successful sperm retrieval in men with azoospermia who are undergoing testicular sperm extraction. Fertil Steril. 2002;78(6):1195–1198. doi:10.1016/S0015-0282(02)04259-0

167. Ballesca JI, Balasch J, Calafell JM, et al. Serum inhibin B determination is predictive of successful testicular sperm extraction in men with non-obstructive azoospermia. Hum Reprod. 2000;15(8):1734–1738. doi:10.1093/humrep/15.8.1734

168. Franco G, Scarselli F, Caccioni V, et al. A novel stepwise micro-TESE approach in non obstructive azoospermia. BMC Urol. 2016;16(1):20. doi:10.1186/s12894-016-0138-6

169. Alrabeeah K, Wachter A, Phillips S, Cohen B, Al-Hathal N, Zini A. Sperm retrieval outcomes with microdissection testicular sperm extraction (micro-TESE) in men with cryptozoospermia. Androl. 2015;3(3):462–466. doi:10.1111/and.12000

170. Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. Androl. 2014;2(1):20–24. doi:10.1111/and.2013.2.issue-1

171. Haliloglu AH, Tangal S, Gulpinar O, Onal K, Pabuçcu R. Should repeated TESE be performed following a failed TESE in men with Klinefelter syndrome? Androl. 2014;2(1):42–44. doi:10.1111/and.2013.2.issue-1

172. Kalsi J, Thum M-Y, Muneer A, Abdullah H, Minhas S. In the era of micro-dissection sperm retrieval (m-TESE) is an isolated testicular biopsy necessary in the management of men with non-obstructive azoospermia? BJU Int. 2012;109(3):418–424. doi:10.1111/bju.12012.109.issue-3