



Male Infertility: Present and Future

¹Indrashis Bhattacharya, ²Subeer S Majumdar

ABSTRACT

During the last decade, a sudden decline in human male fertility has become a global concern. Genetic, endocrine and life style related problems are generally considered to be the underlie causes. However, several cases have been reported where hormones fail to promote spermatogenesis. The reason of such reproductive malfunctioning leading to male infertility remains idiopathic. Therefore, a sincere attention with a coordinated multidisciplinary effort by the andrologists, urologists and basic scientists is needed to understand the regulation of the sperm production. Powerful tools like high through put gene expression analyses, genetically manipulated mouse models and tissue culture or transplantation based assays are now available, increasing the scope of a establishing a special line of therapy for idiopathic male infertility. In this review, we have briefly summarized various categories of male infertility with emphasis on current research advancement in this field.

Keywords: Male infertility, Primary testicular failure, Testicular dysgenesis syndrome, Spermatogenesis.

How to cite this article: Bhattacharya I, Majumdar SS. Male Infertility: Present and Future. MGM J Med Sci 2015;2(1): 16-24.

Source of support: UGC, DBT, ICMR.

Conflict of interest: None

INTRODUCTION

The alarming decline in sperm count with poor understanding about the mechanisms involved is a great challenge for the clinicians for delivering appropriate therapy.¹ Research in the last decade has revealed various aspects of male reproductive health of human, such as the rising incidence of primary testicular failure, testicular cancer, declining semen quality, increasing frequencies of undescended testis, hypospadias, precocious male puberty and ultimately male infertility.² Here, we have briefly discussed these various aspects of reproductive health of human and will highlight the probable cause and remedies.

¹Assistant Professor, ²Staff Scientist-VII and Incharge

¹Department of Zoology, Hemwati Nandan Bahuguna Garhwal University, Srinagar, Uttarakhand, India

²Cellular Endocrinology Laboratory, Embryo Biotechnology Laboratory, Primate Research Center, National Institute of Immunology, New Delhi, India

Corresponding Author: Subeer S Majumdar, Staff Scientist-VII and Incharge, Cellular Endocrinology Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110067 India, Phone: 09818170750, e-mail: subeer@nii.ac.in

Primary Testicular Failure

Testes are the major endocrine gland of the male reproductive system. Endocrine testicular failure results in androgen deficiency. In primary endocrine testicular failure, a decline in testosterone (T) secretion resulting in a condition termed hypoandrogenism is caused by a deficiency or absence of Leydig cell (LC) function. However, the major phenotype of primary exocrine testicular failure is male infertility. Clinically, relevant diseases described here are anorchia, LC hypoplasia, GC aplasia, spermatogenetic arrest, hypospermatogenesis and numerical chromosomal abnormalities as well as Y chromosome microdeletions.²

Congenital Anorchia

Bilateral anorchia is defined as complete absence of testicular tissue in genetically and phenotypically male patients. Bilateral congenital anorchia is rare, the incidence appears to be 1:20,000 males. In unilateral anorchia, testicular tissue is still present on the contralateral side. Unilateral congenital anorchia is about 4 times as frequent. In patients with congenital bilateral anorchia serum gonadotropins are already elevated in childhood and rise to very high levels from the age of puberty onwards. T levels remain within the castrate range. In patients with suspected bilateral anorchia, it is mandatory to rule out cryptorchidism, as cryptorchidism is associated with an increased risk for testicular cancer and should definitely not be overlooked. Both the hCG stimulation test, that examines T secretory capacity, and serum anti-Müllerian hormone (AMH) measurement can be used for differential diagnosis. During hCG administration T levels remain unchanged in patients with bilateral anorchia even after a 7-day period of stimulation while a rise can be detected in patients with cryptorchidism. In comparison to the hCG test, measurement of AMH, which is undetectable in anorchia, has a higher sensitivity, but equal specificity for differentiation of bilateral anorchia from bilateral cryptorchidism. Endocrine tests are not useful for differential diagnosis of unilateral anorchia. In these cases imaging techniques, such as computer tomography or MRT and finally exploratory surgery or laparoscopy have to be applied. Unilateral anorchia does not require therapy. In phenotypically male patients with bilateral



congenital anorchia T substitution has to be implemented at the time of expected puberty. For psychological or cosmetic reasons implantation of testicular prostheses could be offered to the patient although these are often expensive. To date, there is no treatment of infertility in bilateral anorchia.²

Leydig Cell Hyperplasia

Leydig cell hypoplasia is a rare disease with an autosomal recessive pattern of inheritance and estimated incidence of 1:1,000,000. Leydig cells are unable to develop because of inactivating mutations of the LH-receptor (LH-R) that fails to provide the necessary stimulation of intracellular pathways. Men with LC hypoplasia present with very low serum T and high LH levels. The phenotype is dependent on the extent of intrauterine T secretion. Two types of LC hypoplasia have been described.

- Type I is the most severe form, resulting in a female phenotype of the external genitalia with blind ending vagina, primary amenorrhea, and absence of secondary sex differentiation at puberty. It is caused by inactivating mutations in the LH-R that completely prevent LH and hCG signal transduction and thus T production.
- Type II LC hypoplasia is characterized by milder signs of androgen deficiency with hypogonadism with micropenis and/or hypospadias. This milder form is derived from mutations of the LH-R, which only partially inactivate signal transduction and retain some responsiveness to LH. Testicular histology reveals seminiferous tubules, whereas LC are not present or appear only as immature forms. Epididymides and deferent ducts are usually present, whereas the uterus, tubes or upper vagina are not found.²

Spermatogenic Failure

Whereas endocrine testicular failure causes hypogonadism, spermatogenic failure—defined as exocrine testicular failure—leads to male infertility. Spermatogenic failure might be caused by hypothalamic, pituitary, testicular or post-testicular disorders. Various testicular etiologies of spermatogenic failure may lead to the same histopathological pattern. In this sense, GC aplasia, spermatogenic arrest, and hypospermatogenesis have to be considered as a description of certain histopathologic phenotypes of spermatogenic failure, and not as manifestations of single disease entities² (Table 1).

Numerical Chromosomal Abnormalities

Klinefelter syndrome: This syndrome was first described by Harry Klinefelter in 1942 as a clinical condition with small testes, azoospermia, gynecomastia and an elevated

serum FSH. In 1959, there was the chromosomal basis of the disorder described. Subsequently, the diagnosis of Klinefelter syndrome has required the demonstration of the 47, XXY karyotype or one of its rarer variants. The prevalence of Klinefelter syndrome appears to be approximately 1 in 660 males, and recent data suggest a rising incidence over the last decades. It is the most frequent form of primary testicular dysfunction affecting spermatogenesis as well as hormone production and is found in about 12% of men presenting with azoospermia. It appears that at least half of the cases remain undiagnosed and untreated throughout life. A nonmosaic 47, XXY karyotype is found in 80 to 90% of Klinefelter patients. A mosaic is seen in another 5 to 10% of patients. The 47, XXY/46, XY mosaicism is most common. The 48, XXXY, 48, XXYY and 49, XXXXY karyotypes constitute 4 to 5% of all Klinefelter syndrome karyotypes, structurally abnormal extra X chromosomes are found in less than 1% of patients. The numerical aberration in nonmosaic 47, XXY is derived with equal likelihood from maternal or paternal meiotic error. Most cases are caused by meiosis without X/Y or X/X recombination. Advanced maternal age seems to be a risk factor. It is not known whether the 47,XXY karyotype is slightly over-represented among spontaneous abortions and stillbirths. However, in contrast to many other aneuploidies, Klinefelter syndrome seems to be only a minor risk factor and most pregnancies result in a live-birth. In most patients, early stages of puberty proceed normally. Postpubertally, the syndrome is characterized by the small testes and infertility because of azoospermia. Testicular histopathology in adult men with Klinefelter syndrome displays various patterns. Classically, GC aplasia, total tubular atrophy or hyalinizing fibrosis and relative hyperplasia of LC are found. However, in some adult Klinefelter patients, foci of spermatogenesis up to the stage of mature testicular sperm can be detected. T replacement must be considered a lifelong therapy in Klinefelter patients to assure quality of life. Usually, gynecomastia is not influenced by hormone therapy. If it disturbs the patient, a plastic surgeon experienced in cosmetic breast surgery could perform a mastectomy.²

XX-male syndrome: The XX-male syndrome is characterized by the combination of male external genitalia, testicular differentiation of the gonads and a 46,XX karyotype by conventional cytogenetic analysis. This disorder shows a prevalence of 1:9,000 to 1:20,000. Applying molecular methods it has been demonstrated that about 3 of 4 XX-males have Y chromosomal material translocated onto the tip of one X chromosome. Translocation of a DNA-segment which contains the testis-determining gene (SRY: Sex determining

Table 1: Histopathological phenotype

<i>Name</i>	<i>Effect</i>
1. GC aplasia or Sertoli cell only (SCO) syndrome	This is a histopathologic phenotype where the seminiferous tubules are reduced in diameter, and contain only SC but no other cells involved in spermatogenesis. GC aplasia or SCO syndrome is one common cause of nonobstructive azoospermia. In congenital germ cell aplasia, the primordial germ cells do not migrate from the yolk sac into the future gonads or do not survive in the epithelium of the seminiferous tubule. Chromosomal abnormalities, especially microdeletions of the Y chromosome, are important genetic causes for complete germ cell aplasia. Moreover, antineoplastic therapy with radiation or chemotherapy may cause complete loss of GC. Other reasons include viral infections of the testes, such as mumps orchitis. GC aplasia can also occur in maldescended testes.
2. Spermatogenic arrest	This is also not a specific primary testicular failure, but a histopathological description of the interruption of normal GC maturation at the level of a specific cell type including that of spermatogonial arrest, spermatocyte arrest, or spermatid arrest. A definite diagnosis can only be made by multiple testicular biopsies. Testicular volume, FSH and inhibin B may lie in their normal ranges, but may also be elevated or decreased, respectively. The arrest may be caused by genetic or by secondary influences. Genetic etiologies include trisomy, balanced-autosomal anomalies (translocations, inversions) or deletions in the Y chromosome (Yq11). It is important to note that numerous mouse knock out models that display as spermatogenic phenotype, including sperm cell arrest, has contributed little of clinical relevance to the large number of men with idiopathic infertility. The possible role of several gene mutations and polymorphisms has been extensively investigated but no clear-cut genetic factor could be identified so far. An association between increased androgen receptor CAG repeat length (which reduces the transcriptional activity of the androgen receptor) and idiopathic male infertility is suggested by a recent, comprehensive meta-analysis. However, no gene mutation or polymorphism analysis has the necessary sensitivity and specificity to be recommended as a diagnostic tool in the workup of male infertility.
3. Hypospermatogenesis	This is the final and major histological grouping in primary exocrine testicular failure in which all GC types, including mature spermatids, is present in some or all tubules, but are mildly, moderately or severely reduced in number. Some patients have the appearance of complete GC aplasia in some tubules but with complete spermatogenesis in adjacent tubules (sometimes called 'focal' germinal cell aplasia) while others have the appearance of an excess number of precursor GC in relation to the number of mature spermatids in the epithelium. In most patients with hypospermatogenesis testicular volume is reduced. FSH is elevated in most, but not all patients, with serum levels correlating positively with the proportion of tubules with GC aplasia. Several studies have demonstrated that inhibin B is a more sensitive and specific endocrine marker of hypospermatogenesis. However, even the combined measurement of inhibin B and FSH provides no certainty concerning the presence or absence of sperm in multiple testicular biopsies. In azoospermic men with severe hypospermatogenesis, pregnancies can be achieved with testicular sperm extracted (TESE) from testicular biopsies that are injected into mature oocytes by intracytoplasmic sperm injection (ICSI). It has been suggested that residual sperm production could be improved by FSH therapy in incomplete GC aplasia. However, randomized studies performed so far have demonstrated some increase in total sperm count in the ejaculate but failed to prove a significant effect on fertility.

region Y) from the Y to the X chromosome takes place during paternal meiosis. The presence of the gene is sufficient to cause the initially indifferent gonad to develop into a testis. Most SRY-positive patients are very similar to patients with Klinefelter syndrome. In general, however, 46, XX males are significantly shorter than Klinefelter patients or healthy men, resembling female controls in height and weight. The incidence of maldescended testes is significantly higher than that in Klinefelter patients and controls. The testes are small and endocrine changes of primary testicular failure with decreased serum testosterone and elevated estrogen

and gonadotropin levels. About every second patient develops gynecomastia. XX-males seem to have normal intelligence, however, exact data are lacking. Ejaculate analysis reveals azoospermia. The testicular histology of postpubertal SRY-positive XX males shows atrophy and hyalinization of the seminiferous tubules devoid of Gc. For the SRY-negative XX-males (1 of 4 XX-males) the mechanism underlying the sex reversal remains unclear. These patients are less virilized than SRY-positive men and show malformations of the genital organs, such as maldescended testes, bifid scrotum or hypospadias. Today, there is no therapy for infertility of men with



XX-male syndrome. Patients with reduced testosterone production have to receive appropriate testosterone replacement therapy.²

XYY syndrome: Most 47, XYY males have no health problems distinct from those of 46, XY males. The diagnosis relies entirely on the cytogenetic demonstration of two Y chromosomes with an otherwise normal karyotype. The nonmosaic chromosomal aneuploidy is caused by nondysjunction in paternal meiosis. Usually, the finding is incidental, occurring when karyotyping has been undertaken for unrelated issues. The prevalence among unselected newborns appears to be 1:1000. Men with 47, XYY-syndrome have serum levels of T and gonadotropins, as well as testicular volumes, comparable to those of normal healthy men. Most men with 47, XYY-syndrome have normal fertility.²

Structural Chromosome Abnormalities

Structural abnormalities of the autosomes: Balanced autosomal anomalies may interfere with the meiotic pairing of the chromosomes and thus adversely affect spermatogenesis. These abnormalities often do not display a typical clinical phenotype. The presence and extent of disturbed fertility cannot be foreseen in individual cases. The same balanced autosomal aberration can have a severe effect on spermatogenesis in one patient and none at all in another patient. Even brothers with the same pathological karyotype can have widely differing sperm densities. So far, no clinical or laboratory parameter in an infertile male is known which reliably indicates the presence of an autosomal structural anomaly. Therefore, in cases of unclear azoospermia or severe oligozoospermia karyotyping is generally advised.²

Structural Abnormalities of Sex Chromosomes

X chromosome: The X chromosome contains numerous genes essential to survival. Every major deletion of this chromosome has a lethal effect in the male sex. Translocations between the X chromosome and an autosome usually result in disturbed spermatogenesis, whereas inversions of the X chromosome do not substantially affect male fertility.

Y chromosome: The intact Y chromosome is essential for the male reproductive system. The male-specific region of the Y chromosome (MSY) differentiates the sexes and comprises 95% of the chromosome length. The SRY gene is localized on the short arm of the Y chromosome and it influences differentiation of the embryonic gonad in the testicular pathway. The long arm of the Y chromosome contains areas responsible for the regular

spermatogenesis. Short arm deletions of the Y chromosome that encompass the sex determining SRY gene result in sex reversal. Clinically, affected men appear as phenotypically female individuals with somatic signs of Turner's syndrome. If the deletion affects the long arm, the phenotype will be male. Loss of the heterochromatic part of the Y chromosome's long-arm (Yq12) leaves general and reproductive health unaffected. Deletions of the euchromatic part of the Y chromosome's long arm (Yq11) may affect spermatogenesis, because Yq11 harbours loci essential for spermatogenesis. In addition to deletions, a series of further structural anomalies of the Y chromosome are known. Pericentric inversions are without consequence. An isodicentric Y chromosome is a more complex aberration nearly always occurring as a mosaic with a 45, X-cell line. The phenotype may be male, female or intermediate. Patients with a male phenotype are usually infertile. These patients have an increased risk developing testicular tumors. Reciprocal translocations between the Y chromosome and one of the autosomes are rare. In most cases, spermatogenesis is severely disturbed, however, several men with this karyotype are fertile. Translocations between the X and Y chromosomes occur in several variations; often the karyotype is unbalanced. The correlation between karyotype and clinical presentation is complex. The phenotype may be male or female, fertility may be normal or disturbed.

Y chromosome microdeletions: The long-arm of the Y chromosome contains four partially overlapping but discrete regions that are essential for normal spermatogenesis. The loss of one of these regions, designated as AZF (azoospermia factor) a, P5/proximal P1 (AZFb), P5/distal P1 (AZFb) and AZFc (or b2/b4), can lead to infertility. The deleted regions are usually of submicroscopic dimensions and are known as Y chromosome microdeletions. Their prevalence in azoospermic men lies between 5 and 10% and between 2 and 5% in cases of severe oligozoospermia. Clinically, the patients present with severely disturbed spermatogenesis; endocrine testicular function may or may not be affected by the microdeletion as in other cases of spermatogenetic failure. Testicular histopathology varies from complete or focal Sertoli-cell-only pattern to spermatogenic arrest or hypospermatogenesis with qualitatively intact but quantitatively severely reduced spermatogenesis. In azoospermic men, the presence of a complete deletion of AZFa or AZFb seems to be associated with uniform GC aplasia and negative prognostic value for testicular sperm retrieval. No clinical parameter can help distinguishing patients with microdeletions of the Y chromosome from infertile men without microdeletion. It should be noted that Y chromosome microdeletions have also been described in proven fertile men.²

Table 2: Causes and frequencies of male infertility

Sl. no.	Abnormality	Effect	Frequency (%)
1	Chromosomal aberrations		
	Klinefelter's syndrome 47, XXY	Azoospermia to severe oligospermia	5-10
	Other sex chromosome alterations	Azoospermia to normospermia	0.1-0.2
	Robertsonian/reciprocal translocations	Azoospermia to severe oligospermia	0.5-1
2	Y chromosomal microdeletions		
	AZFa	Azoospermia to Sertoli cell syndrome	0.5-1
	AZFb	Azoospermia to spermatogenic arrest	do
	AZFc	Azoospermia to severe oligospermia	3-7
	AZFb + c	Sertoli cell syndrome/spermatogenic arrest	0.5-1
	Partial AZFc deletion	Azoospermia to normospermia	3-5
3	Gene mutation		
	CFTR	Obstructive azoospermia	60-70
	AR	Azoospermia to oligospermia	2-3
	INSL3-LGR8	Cryptorchidism	4-5
4	Hypogonadotropic hypogonadism		
	Congenital: Kallmann syndrome	Azoospermia	0.5
	Acquired: tumor, infection, autoimmune, pituitary infarction and drug use	Azoospermia to oligospermia	0.25
5	Hypergonadotropic hypogonadism		
	Classic: Klinefelter's syndrome	Azoospermia to severe oligospermia	5-10
	Oligozoospermia	Azoospermia to severe oligospermia	0.5-0.25

Table 2 describes the frequencies of all such pathophysiological conditions leading to male infertility.

Testicular Dysgenesis Syndrome

Several lifestyle (obesity, smoking) and environmental-related (exposure to traffic exhaust fumes, dioxins, combustion products, pesticides, food additives, persistent pollutants, such as DDT, polychlorinated biphenyls) factors have appeared to be negatively associated with both the perinatal and adult testes, emphasizing the importance of environmental/lifestyle impacts throughout the life course. Such observations have recently³ given rise to this testicular dysgenesis syndrome (TDS) hypothesis which proposes that each of these disorders may have a common fetal origin triggered by maldevelopment of the testis, leading to malfunction testes. We will now summarize these new aspects of poor semen quality, testis cancer, undescended testis and hypospadias are symptoms of one underlying entity, TDS, which is increasingly common due to adverse environmental influences.

Testicular Germ Cell Cancer

Germ cell proliferate and differentiate normally during fetal life and in the postnatal period to facilitate normal spermatogenesis in adulthood. However, GC differentiation in perinatal life and the regulatory processes involved are poorly understood. Based on rodent studies, there are three important steps of this process (as discussed earlier in the text see Biology of Spermatogenesis section). At first, the fetal GC lose their pluripotency, by expressing a number of differentiating markers shared with embryonic stem

cells (e.g. OCT4). Failure to execute this differentiation step results in persistence of pluripotency characteristics, which is dangerous as such cells can potentially differentiate into various tissues. Such a failure is thought to be the underlying mechanism via which carcinoma *in situ* (CIS) cells are formed in the human, and it is from these CIS cells that testicular germ cell cancer (TGCC) subsequently develops in young adulthood incidence of TGCC has increased progressively in many Western nations over the past 60 years, and among Caucasians is the commonest cancer of young men. This increase is too rapid to be explained by genetic factors and is, therefore, presumed to have environmental/lifestyle causes. This is reinforced by studies of migrants from a country with a high incidence of TGCC (e.g. Denmark) to a country with a lower incidence (e.g. Finland, Sweden). Risk of TGCC in migrant males is comparable to their country of origin, but incidence of risk in children born in the country to which their parents have emigrated is the same as in that country. The causes of the increase in TGCC and the between-country differences are unexplained, but differential exposure to environmental chemicals (ECs) has been suggested as one of the possibilities. This includes (i) Danish men born during the Second World War had a lower frequency of testicular cancer in adulthood than expected from the overall incidence of the disease, and (ii) a strong association between low-birth weight and testicular cancer.

Cryptorchidism and Hypospadias

Other disorders of male reproductive development, such as cryptorchidism (undescended testis) and hypospadias



(birth defect of urethra), are associated with increased risk of TGCC, and individuals with low sperm counts/quality and, therefore, infertility in adulthood are also at increased risk of TGCC. Studies have showed that cryptorchid testis is associated with some degree of maldevelopment of the seminiferous tubules followed by primary testicular failure (as we have discussed earlier in this section), including Sertoli-cell-only syndrome and spermatogenic arrest. Moreover, the association of cryptorchidism with testicular cancer is also well documented. Since, undescended testis is an usual phenomenon at the time of birth, the condition is, per definition, of fetal origin, which again is in line with reports showing a strong association between low birth weight and the testicular maldescent. Although the molecular mechanisms behind the recent rise in the incidence of cryptorchidism are not known, the influence of environmental factors is a plausible hypothesis.

Testicular Dysgenesis Syndrome and Environment

Testicular dysgenesis syndrome may be caused by genetic or environmental factors, or a combination of both. The advent of modern molecular genetics has expanded the understanding of genetics of endocrine disorders, including dysgenesis of the testis, which may be caused by a range of genetic defects. However, in a significant number, perhaps the majority, of newborns with malformations of genitalia, no chromosomal or other genetic defect can be demonstrated with our current knowledge. These cases should be subject to exploration for possible environmental etiology. The relevance of considering a possible role of environment factors for development of TDS is highlighted by epidemiological findings of geographic and temporal synchrony in the symptoms of TDS. As stated earlier, the rates of testis cancer, undescended testis and hypospadias are much lower in Finland than Denmark.

Effects of maternal lifestyle/environment on son's testes: The incidence of female obesity has increased considerably, and one preliminary study has suggested that high maternal BMI negatively affects semen quality in resulting sons when they grow to adulthood, with indirect evidence pointing toward reduced SC number. Over the same time period, exposure to a wide range of environmental chemicals (ECs) has increased, several of which are clearly documented to have intrinsic endocrine-disrupting activity, in particular anti-androgenic activity. The so-called 'estrogen hypothesis' has been expanded to include also environmental anti-androgens as endocrine disrupters with potential adverse effect on male reproductive health. For example, (i) many of the persistent organochlorine compounds, including various

pesticides and polychlorinated biphenyls (PCBs), are lipophilic and accumulate in fat. Exposure of a range of persistent ECs (e.g. PCBs, polybrominated compounds or certain phthalates) present in cosmetics/toiletries/medications during pregnancy are known to increase the risk of TDS disorders human male offspring in adulthood. (ii) Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a highly toxic by-product of combustion interact with the aryl hydrocarbon (AH) receptor, and a number of other ECs also interact with this receptor, such as polycyclic aromatic hydrocarbons (PAHs) that are constituents of exhaust fumes, smoke and cooking processes. Human exposure during pregnancy to dioxin as a result of the Seveso accident in 1976 resulted in lower sperm counts in 'exposed' (fetal) males in adulthood whereas those who were exposed to dioxin as adult men showed no effect. (iii) Substantial reductions in sperm counts (approx. 40% in three studies) were found in men whose mothers had smoked heavily in pregnancy. Whatever the explanation, the initial trigger will probably have involved interaction of PAHs (or other constituents) in the cigarette smoke with the Ah receptor, although other mechanisms are possible. A interesting finding from studies involving exposure of fetal rats to anti-androgenic chemicals during specific fetal time windows is that exposure during what is termed the 'masculinization programming window' is associated in adulthood with reduced testicular size and presumably therefore reduced sperm production, although the basis for this relationship has yet to be established as it does not appear to involve a straightforward relationship with SC number. It is important to note that, although all these human male reproductive problems currently of concern in relation to environmental hazards can be experimentally produced in rodents by pre- and perinatal exposure to endocrine disrupters—with the exception of GC cancer, for which, unfortunately, there is no suitable animal model as yet.

Obesity

An important lifestyle-dependent factor that adversely affects spermatogenesis is obesity. Several studies have shown-up to a threefold higher incidence of obesity in infertile men than in those with normal semen quality, a BMI of more than 25 is associated with an average 25% reduction in sperm count and sperm motility. Obesity in men is associated with reduced blood T levels, this reduction being proportional to the degree of obesity. Another explanation for reduced spermatogenesis in obese men could be deposition of fat around the scrotal blood vessels, leading to impaired blood cooling and elevated testicular temperature.

Smoking, Alcohol and Drugs

Smoking and alcohol consumption are two major lifestyle factors that have adverse effects on male reproductive health. The effects of recreational, sports (anabolic steroids) and therapeutic drugs on spermatogenesis in men are also reported. Administration of androgenic steroids to men results in reduced spermatogenesis because it causes suppression of LH secretion from the pituitary gland and a resulting suppression of intratesticular T levels; this has been widely evaluated as an approach to male contraception. Some chemotherapeutic agents (anti-mitotics, such as cyclophosphamide) used for treatment of cancers or of some kidney diseases have well-documented adverse effects on spermatogenesis and/or fertility. Studies indicate reduced fertility in epileptic men and one explanation is that the drugs used for treatment (carbamazepine, oxcarbazepine, valproate) are associated with adverse effects on sperm number, morphology or motility.

Scrotal Heating and Sedentary Position

Testicular descent into the scrotum normally occurs by birth in boys and failure of testicular descent (cryptorchidism), especially when this extends into puberty and adulthood, results in absence of spermatogenesis. The testes descend into the scrotum in order that their temperature can be kept 3 to 4°C below core body temperature, as maintenance at normal body temperature is incompatible with spermatogenesis. Heat exposure causes hypoxia and oxidative stress responses in the GC, manifest as increased expression of hypoxia inducible factor 1 α , hemoxygenase 1, glutathione peroxidase 1 and glutathione-S-transferase- α , which induce GC apoptosis. Lifestyle and occupational factors that cause men to spend a long time in a sedentary position, something that has become common for many men working in today. For example, studies in lorry and taxi drivers, who spend a long time seated, have also produced evidence for detrimental effects on semen quality.

Environmental Exposure to Pollutants

Exposure to ozone as the result of general atmospheric pollution has been negatively associated with sperm counts in a large group of sperm donors. The environmental pesticide exposures via fruit and vegetables or the general environment can adversely affect spermatogenesis in men at large. Exposure to organochlorine pesticides (e.g. DDT) that have been banned in western countries, as there will no longer be occupational exposure to such compounds, whereas there is exposure of the general population because of their persistence in the environment and food chain. There are one or

two well-documented examples of occupationally induced infertility resulting from EC exposure, the best known being that of dibromochloropropane (DBCP), a nematocide used on crops such as bananas and pineapple. Exposure of men to DBCP during manufacture or application caused severe impairment of spermatogenesis and resulting infertility in a high proportion of highly exposed men. Other occupational exposures that have been shown in epidemiological studies to negatively affect spermatogenesis and/or sperm counts in men include inorganic lead and other heavy metals (cadmium, mercury arsenic), metal welding fumes and carbon disulphide. However, overall, the present view is that there is no firm evidence that exposure of adult men to common ECs, whether persistent or not, has any major impact on their fertility or semen quality although further studies of perfluorinated chemicals (alachlor, atrazine and diazinon) are warranted.

Precocious Male Puberty and Testotoxicosis

Testotoxicosis is a form of gonadotropin-independent precocious puberty in which boys experience early onset and progression of puberty. Patients have accelerated growth, early development of secondary sexual characteristics and usually reduced adult height. Testotoxicosis is caused by an activating mutation of the LH-R, leading to increased levels of sex steroids in the context of low LH. Therapy has, therefore, traditionally targeted steroidogenesis. However, the drugs used have been associated with side effects. More recently, a combination of an oral anti-androgen spironolactone and an aromatase inhibitor testolactone decreased height velocity and improved predicted height. A phase II study in testotoxicosis is currently underway, exploring the combination of a highly selective anti-androgen, bicalutamide, and the potent aromatase inhibitor, anastrozole. These agents are well tolerated in the populations in which they have been studied and effectively inhibit testosterone activity and estrogen production, in adult patients.²

Male Infertility

About 15% couples worldwide are childless due to infertility. Sixty-one countries are facing fertility related problems at or below the replacement level. Fifty percent of such infertility is because of the male factor and of which half is idiopathic in nature.¹ The cornerstone of diagnosis for males is the routine semen analysis in which sperm concentration, motility, morphology and the presence of other cells (spermatogenic and white blood cells) are assessed, as well as indicators of the patency and function of the genital tract (volume, liquefaction time, pH and fructose presence or absence) (Table 3).



Table 3: Sperm count and quality

Name	Description
Azoospermia	Complete absence of sperm
Aspermia	Ejaculation does not emit any semen
Oligozoospermia	<10 million sperm/ml of semen
Asthenozoospermia	>40% of sperm have low motility
Teratozoospermia	>40% of sperm with abnormal morphology
Necrospermia	Nonviable/dead sperm
Oligoasthenozoospermia	Motile density <8 million sperm/ml
Hemospermia	Red blood cells present in semen
Pyospermia	White blood cells present in semen
Polyzoospermia	Excessively high sperm concentration

The semen analysis, however, is not a test of fertility potential and indicates infertility only in the case of azoospermia (absence of sperm in the ejaculate; non-obstructive and obstructive). The majority of endocrine disorders are relatively simple to diagnose and treat because of the availability of tests to diagnose endocrine defects. However, it is important to note that in patients suffering from such male infertility, hormonal therapy fails to initiate spermatogenesis.⁶ In past decade, several mouse models have identified hundreds of proven candidate male infertility associated genes.^{1,4,5} Due to lack of a rigorous clinical evaluation, these etiologies are not diagnosed properly. Therefore, it is high time to study the biology of the individual cells present inside the seminiferous epithelium.

New Advancements and Approaches to treat Male Infertility

Male GC Transplantation

In 1994, GC transplantation was developed by Dr Ralph Brinster et al at the University of Pennsylvania. These ground-breaking studies demonstrated that microinjection of GC from fertile donor mice into the seminiferous tubules of infertile recipient mice results in donor-derived spermatogenesis and sperm production by the recipient animal. The use of donor males carrying the bacterial β -galactosidase gene allowed identification of donor-derived spermatogenesis and transmission of the donor haplotype to the offspring by recipient animals. Surprisingly, after transplantation into the lumen of the seminiferous tubules, transplanted GC were able to move from the luminal compartment to the basement membrane where undifferentiated spermatogonia are located. It is generally accepted that only spermatogonial stem cells (SSCs) are able to colonize the niche and re-establish spermatogenesis in the recipient testis.

Therefore, GC transplantation provides a functional approach to study the stem cell niche of the testis and to characterize putative SSC. To date, GC transplantation is used to elucidate basic stem cell biology, to produce transgenic animals through genetic manipulation of GC prior to transplantation, to study SC-GC interaction, SSC homing and colonization as well as SSC self-renewal and differentiation.⁶

Xenotransplantation of Testicular Tissue

In 2002, Dobrinski et al demonstrated that small fragments of testis tissue from immature males transplanted under the dorsal skin of immunodeficient mice are able to survive and undergo full development with the production of fertilization competent sperm. Since, then testicular tissue xenografting has been shown to be successful in many other species and emerged as a valuable alternative (other than GC transplantation) approach to understand testis development and spermatogenesis of larger animals including human in mice.⁷

In vitro Generation of Sperm Capable of Fertilization

Studies on *in vitro* spermatogenesis date back to organ culture experiments about a century ago. In 1937, it was reported that spermatogenesis proceeded up to the pachytene stage of meiosis in testis tissues of newborn mouse placed on a clot. In the 1960s, organ culture methods had advanced and various conditions were extensively examined. However, it was not possible to promote spermatogenesis beyond the pachytene stage. Thereafter, cell culture methods, instead of organ culture, were used with new concepts and devices, including immortalized GC lines, the production of SC lines as feeder cells, bicameral chamber methods, etc. Despite of such endeavors, it has not been impossible to produce fertility-proven haploid cells from spermatogonial stem cells *in vitro*. In 2011, Takehiko Ogawa et al in Yokohama University, Japan have demonstrated that neonatal mouse testes which contain only gonocytes or undifferentiated spermatogonia as the only representative GC can produce spermatids and sperm *in vitro* with serum-free culture media. Spermatogenesis was maintained over 2 months in tissue fragments positioned at the gas-liquid interphase. The obtained spermatids and sperm resulted in healthy and reproductively competent offspring through microinsemination. In addition, neonatal testis tissues were cryopreserved and, after thawing, showed complete spermatogenesis *in vitro*. This organ culture method developed by the team has been shown to be applicable through further refinements to a variety of

mammalian species, which will serve as a platform for future clinical application as well as mechanistic understanding of spermatogenesis. Moreover, this group has further developed a new *in vitro* system that can induce full spermatogenesis from SSC lines or any isolated SSC. This method depends on an organ culture system onto which SSC are transplanted. The settled SSC form colonies and differentiate up into sperm. The resultant haploid cells are fertile, and give rise to healthy offspring through micro-insemination. Thus, these two *in vitro* culture systems are unambiguously the major advancement in the field of sperm biotechnology in the past 70 years of research and potentially create a strong hope for the upcoming challenge of human infertility.⁸

Functional Genomics and Differential Gene Expression

Researchers have initiated evaluating the status of genes expressed during spermatogenically active and inactive conditions of the testis by using differential genomics like differential display to high throughput cDNA microarray and next generation sequencing. Additionally, *in vivo* mouse models are being generated using transgenic or knock out/down technologies for such candidate genes to explore its functionality further as described in details by us before.^{4,5,9}

FINAL CONCLUDING REMARKS

Genetic, physiological and lifestyle factors are known to affect male fertility. Male infertility and declining sperm count may pose a major challenge for human race. However, use of modern tools to undertake cellular and molecular analyses of such situation and studies of functional genomics to determine candidate genes responsible for a particular phenotype may potentially help in understanding causes of such disabilities and

remedies thereof. Gene therapy and stem cell-based therapy, though in infancy, may also contribute in future to reconstitute lost fertilizing potential of the males suffering from idiopathic infertility.

ACKNOWLEDGMENT

The authors are grateful to the past and present Directors of NII and the Head, Department of Zoology, HNB Garhwal University, for constant encouragement. Our thanks to DBT/ICMR and UGC, for the financial support.

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