CHAPTER 6 – PRIMARY TESTICULAR FAILURE

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Abstract

Primary testicular failure may result in endocrine failure, leading to testosterone deficiency or exocrine failure causing impaired spermatogenesis and subsequently male infertility. While some aspects of primary testicular failure are described in detail in separate chapters of Endotext.com, this chapter focuses on congenital or acquired anorchia, Leydig cell hypoplasia, and spermatogenic failure including germ cell aplasia (Sertoli cell only syndrome), spermatogenic arrest, hypospermatogenesis, and mixed atrophy. In addition, genetic causes for primary testicular failure are described such as numerical chromosome aberrations including Klinefelter syndrome, XX-Male syndrome, and XYY syndrome, structural chromosome aberrations of the autosomes or sex chromosomes, and Y chromosome microdeletions. For complete coverage of this and related areas in Endocrinology, please visit our free web-book, www.endotext.org.

Key messages

- Bilateral anorchia is defined as a complete absence of testicular tissue in genetically and phenotypically male patients.
- Leydig cells hypoplasia is caused by inactivating mutations of the LH receptor.
- Primary spermatogenic failure has to be considered as a description of certain histopathologic phenotypes, and not as a manifestation of single disease entities.
- Several genetic causes for primary spermatogenic failure have been elucidated recently.
- Modern management of patients with primary testicular failure caused by numerical chromosome aberrations such as Klinefelter syndrome can ameliorate symptoms of testosterone deficiency and – at least in some patients – can overcome infertility.
- Up to date clinical guidelines are available for molecular diagnosis of Y chromosome microdeletions.
- Novel technologies such as whole-genome sequencing will help to greatly increase the fraction of men suffering from primary testicular failure with a clear genetic diagnosis.

INTRODUCTION

The testis has an endocrine as well as an exocrine function. **Endocrine testicular failure** results in testosterone deficiency. In primary endocrine testicular failure, a decline in testosterone secretion (resulting in a condition termed hypoandrogenism) is caused by a deficiency or absence of Leydig cell function. Clinically relevant diseases described in this chapter are anorchia, Leydig cell hypoplasia and numerical chromosome abnormalities. Testicular dysgenesis is another cause for primary testicular failure that is described in depth in Endotext.com, Pediatric Endocrinology, Chapter 7: Sexual Differentiation. In contrast to primary endocrine testicular failure, secondary endocrine testicular failure is caused by absent or insufficient bioactivity of GnRH or LH (see Endotext.com, Endocrinology of Male Reproduction, Chapter 5: Hypogonadotropic hypogonadism and gonadotropin therapy).

The phenotype of primary **exocrine testicular failure** is male infertility. A comprehensive review on causes and treatment of male infertility is given in Endotext.com, Endocrinology of Male Reproduction, Chapter 7: Clinical management of male infertility. Cryptorchidism as a clinically relevant cause for primary exocrine testicular failure is discussed in Endotext.com, Endocrinology of Male Reproduction, Chapter 19: Cryptorchidism and hypospadias and testicular tumors as a cause and/or sequelae of testicular failure is discussed in Endotext.com, Endocrinology of Male Reproduction, Chapter 13: Testicular cancer pathogenesis, diagnosis and endocrine aspects.

This chapter focuses on anorchia, germ cell aplasia, spermatogenetic arrest, hypospermatogenesis, numerical chromosome abnormalities, structural chromosomal abnormalities, as well as Y chromosome microdeletions causing primary exocrine testicular failure.

ANORCHIA

Bilateral anorchia is defined as complete absence of testicular tissue in genetically and phenotypically male patients. In unilateral anorchia testicular tissue is still present on the contralateral side.

Pure anorchia has to be differentiated from conditions with ambiguous and intersex genitalia (see Endotext.com, Pediatric Endocrinology, Chapter 7: Sexual Differentiation). A clinically important differential diagnosis is cryptorchidism and testicular atrophy where testicular tissue is still detectable (see Endotext.com, Endocrinology of Male Reproduction, Chapter 19: Cryptorchidism and hypospadias).

Congenital Anorchia

Bilateral congenital anorchia is rare; the incidence appears to be 1:20,000 males. Unilateral congenital anorchia is about 4 times as frequent.

As male differentiation of the genital tract and development of the penis and scrotum is dependent on the production of anti-Mullerian hormone (AMH) and androgens, the testis must have disappeared after initial activity in cases of bilateral anorchia. For the development of Wolffian duct structures, an ipsilateral testis must be present at least up to the 16th week of gestation ("the

vanishing testis syndrome") (1). Intrauterine infarction of a maldescended testis or testicular torsion appears to be the major contributor to anorchia (2).

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. Testosterone 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 definitively not be overlooked (see Endotext.com, Endocrinology of Male Reproduction, Chapter 13: Testicular cancer pathogenesis, diagnosis and endocrine aspects). Both the hCG stimulation test, that examines testosterone secretory capacity, and serum AMH measurement can be used for differential diagnosis. During hCG administration testosterone 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 (3). 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 (4; 5). 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, testosterone substitution has to be implemented at the time of expected puberty. For psychological or cosmetic reasons, implantation of testicular protheses could be offered to the patient although these are often expensive. To date, there is no treatment of infertility in bilateral anorchia.

Acquired Anorchia

Surgical removal of both testes in patients with androgen-dependent prostate carcinoma is the most prevalent cause for bilateral acquired anorchia. Other reasons include unintended removal or devascularisation during herniotomy, orchidopexy or other testicular surgery, testicular infarction, severe trauma and self-mutilation. If only one testis is lost then fertility and testosterone production will normally be maintained by the remaining testis and no specific therapy is required. However, patients with a single testis require careful management when surgery is planned on the remaining testis.

The clinical appearance in patients with bilateral acquired anorchia depends on the time when testicular loss occurred. Acquired anorchia before puberty leads to the characteristic phenotype of male eunuchoidism and after puberty to the phenotype of post-pubertal testosterone deficiency (see Endotext.com, Endocrinology of Male Reproduction, Chapter 2: Androgen physiology, pharmacology and abuse).

Untreated acquired bilateral anorchia seems to have no effect on life expectancy, but clearly has an adverse effect on the quality of life (6). If both testes have been removed for therapeutic purposes, e.g. in a patient with prostate carcinoma, androgen supplementation is contraindicated. All other patients have to receive permanent testosterone substitution from the time of the expected onset of puberty in order to induce pubertal development, and in an adult immediately after testicular loss to maintain the various androgen-dependent functions.

LEYDIG CELL HYPOPLASIA

Leydig cell hypoplasia is a rare disease with an autosomal recessive pattern of inheritance and estimated incidence of 1:1,000,000. The Leydig cells are unable to develop because of inactivating mutations of the LH receptor that fails to provide the necessary stimulation of intracellular pathways. The underlying gene defect in Leydig cell hypoplasia was first described by Kremer et al (7) and various other defects have since been described (8–21). Men with Leydig cell hypoplasia present with very low serum testosterone and high LH levels. Leydig cell hypoplasia belongs to the group of the disorders of sex differentiation (DSD) and is currently classified as 46,XY DSD.

The phenotype is dependent on the extent of intrauterine testosterone secretion. Two types of Leydig cell 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 receptor that completely prevent LH and hCG signal transduction and thus testosterone production. Leydig cell hypoplasia type II is characterized by milder signs of androgen deficiency with a predominantly male habitus but signs of hypogonadism with micropenis and/or hypospadia. This milder form is derived from mutations of the LH receptor, which only partially inactivate signal transduction and retain some responsiveness to LH (16). Testicular histology reveals seminiferous tubules, whereas Leydig cells 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. In a patient with Leydig cell hypoplasia type II lacking exon 10 of the LH receptor, maternal hCG synthesized during pregnancy probably led to the development of a normal male phenotype, whereas LH was unable to stimulate the mutant receptor at the time of puberty (22; 23). HCG treatment of this patient was capable of inducing testosterone biosynthesis and complete spermatogenesis (22). This case, however, represents an exception. Therapy of 46,XY DSD with complete feminization requires both orchidectomy because cryptorchid gonads are prone to malignant degeneration, and estrogen substitution therapy.

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, or testicular disorders. A comprehensive review on causes and treatments of male infertility is given in Endotext.com, Endocrinology of Male Reproduction, Chapter 7: Clinical management of male infertility. Various testicular etiologies of spermatogenic failure may lead to the same histopathological pattern. In this sense, spermatogenic failure such as germ cell aplasia (Sertoli cell only syndrome), maturation arrest (MA) at different levels of early round spermatids, primary spermatocytes, or spermatogonia, and hypospermatogenesis have to be clearly differentiated from normal spermatogenesis. A key point is that primary spermatogenic failure has to be considered as a description of certain histopathologic phenotypes, and not as a manifestation of single disease entities.

GERM CELL APLASIA (SERTOLI CELL ONLY SYNDROME)

Germ cell aplasia or Sertoli cell only syndrome (SCO) is a histopathologic phenotype that was first described by Del Castillo et al. in 1947 (24). In complete germ cell aplasia the tubules are reduced

in diameter, and contain only Sertoli cells but no other cells involved in spermatogenesis [Figure 1]. Germ cell aplasia can also be focal with a variable percentage of tubules containing germ cells, but in these tubules spermatogenesis is often limited in both quantitative and qualitative terms (25), and such cases should be referred to as hypospermatogenesis (see below). Germ cell aplasia or SCO is one common cause of non-obstructive azoospermia (NOA).

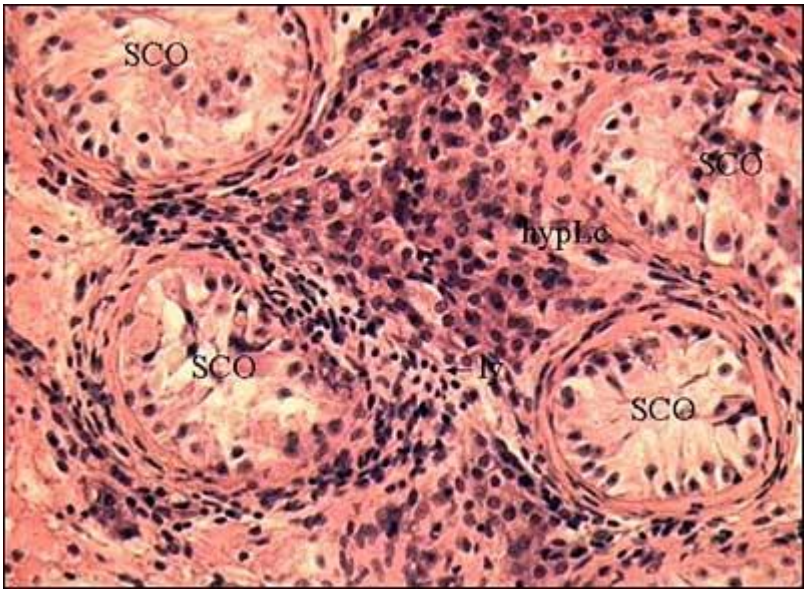

Figure 1. Germinal cell aplasia or Sertoli cell only Syndrome: Seminiferous tubules exhibit only Sertoli cells (SCO). Note the thickening of the lamina propria, focal hyperplasia of Leydig cells (hypLc) and interstitial infiltration of lymphocytes (ly). Primary magnification, x 20.

The lamina propria of SCO tubules is often found to be thickened due to increased collagen type IV and increased thickness of the basal lamina. The latter is associated with an overabundance of the beta2 chain of laminin and thought to be related to spermatogenic dysfunction (26).

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. Anti-neoplastic therapy with radiation or chemotherapy may cause complete loss of germ cells. Other reasons include viral infections of the testes such as mumps orchitis. Germ cell aplasia can occur in maldescended testes.

Chromosomal abnormalities, especially microdeletions of the Y chromosome, are important genetic causes for complete germ cell aplasia (27). These deletions have been characterized and deletions in the AZFb or AZFb+c regions were identified to be important genetic causes of SCO and/or MA resulting in azoospermia (28). Sertoli cells, although showing normal histology, have an increased apoptotic index (29–31).

Several studies tried to identify other genetic risk factors which are associated with SCO. SEPTINS belong to a family of polymerizing GTP-binding proteins being required, for example, for membrane compartmentalization, vesicle trafficking, mitosis and cytoskeletal remodeling. SEPTIN12 participates in male infertility, especially SCO. Although no mutations were found in patients with SCO, 8 coding single-nucleotide polymorphisms (SNP1-SNP8) could be detected in these patients

and the genotype and allele frequencies in SNP3, SNP4, and SNP6 were notably higher than in the control group (32). Most recently, Miyamoto et al. (33) analyzed the human LRWD1 gene whose translated protein mediates the origin recognition complex in chromatin which is critical for chromatin organization in post-G1 cells. Again, no mutations in SCO patients were found, but allele frequencies of two of three SNPs (SNP1 and SNP2) were notably higher compared to controls.

A hint to genetic risk factors leading to SCO was given by Tüttelmann et al. (34). They evaluated copy number variants (CNVs) in patients with severe oligozoospermia and Sertoli-cell-only syndrome and found that sex-chromosomal CNVs were significantly overrepresented in patients with SCO.

Diagnosis of germ cell aplasia can only be made by testicular biopsy. However, the testicular biopsy may not be representative in certain patients, as testicular sperm have been retrieved by testicular sperm extraction (TESE) in patients with apparently "complete germ cell aplasia" following a diligent review of the testicular histology (35). In addition, it has been demonstrated in a large consecutive series of bilateral biopsies from 534 infertile men that a marked discordance of spermatogenic phenotype pattern between both testes can be detected in about 28% of patients (36). Therefore, multiple testicular biopsies of both testes must be scrupulously screened before a diagnosis of complete germ cell aplasia can be made (37).

Patients with the complete form of germ cell aplasia are always azoospermic. Currently, there is no therapy for exocrine testicular failure of patients with complete germ cell aplasia. In general, testosterone production in the Leydig cells is not affected and patients are normally androgenized, and only few patients have hypoandrogenism requiring treatment.

Some patients have the appearance of complete germinal cell 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 germ cells in relation to the number of mature spermatids in the epithelium. Such cases have been described as incomplete or focal germinal cell aplasia which implies, perhaps falsely, a commonality between these disorders and those with complete germinal cell aplasia in all tubules.

SPERMATOGENIC ARREST

Spermatogenic arrest is also not a specific diagnosis for primary exocrine testicular failure, but a histopathological description of the interruption of normal germ cell maturation [Figure 2] at the level of a specific cell type including that of spermatogonial arrest [Figure 3], spermatocyte arrest [Figure 4], and spermatid arrest [Figure 5]). Sometimes, seminiferous cords/nodules with immature Sertoli cells can be found. These Sertoli cells still exhibit anti-muellerian hormone expression indicating their prepubertal state of differentiation. A definite diagnosis can only be made by multiple testicular biopsies.

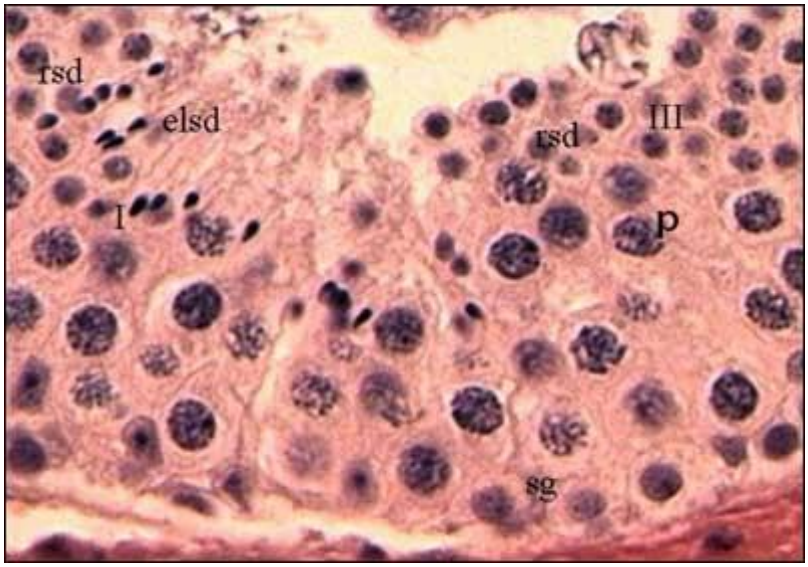

Figure 2. Normal spermatogenesis: Seminiferous epithelium in stage I (I) and stage III (III) of spermatogenesis showing spermatogonia (sg), pachytene spermatocytes (p), round step 1 and 3 spermatids (rsd) and elongating step 7 spermatids (elsd). Primary magnification, x 40.

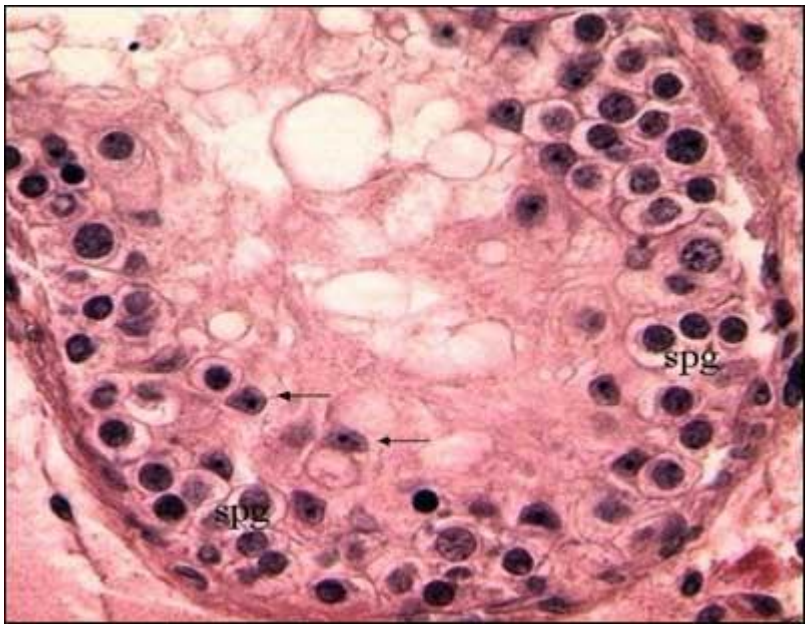

Figure 3. Arrest of spermatogenesis: Seminiferous tubule showing arrest of spermatogenesis at the level of spermatogonia. Note multilayered spermatogonia (spg). Arrow: Sertoli cell nuclei. Primary magnification, x 40.

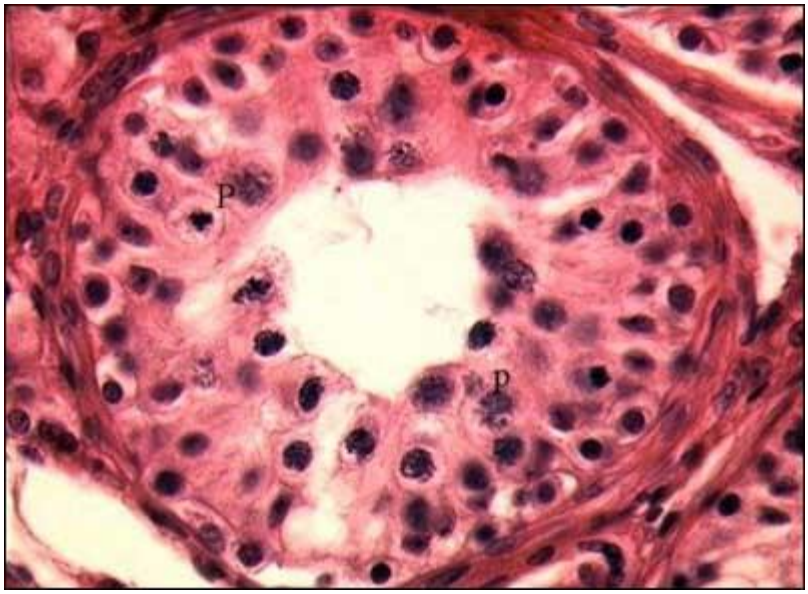

Figure 4. Arrest of spermatogenesis: Seminiferous tubule showing arrest of spermatogenesis at the level of primary spermatocytes in pachytene stage (p). Primary magnification, x 40.

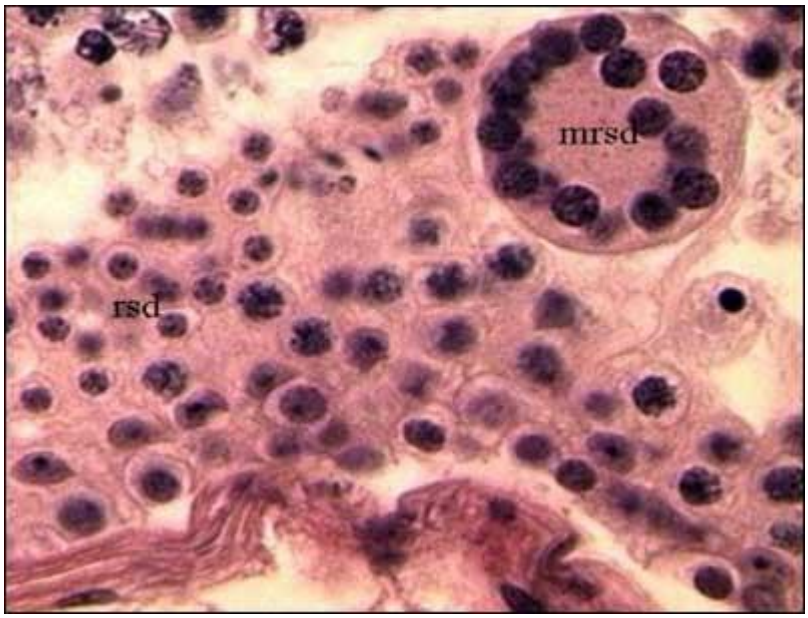

Figure 5. Arrest of spermatogenesis: Seminiferous tubule showing arrest of spermatogenesis at the level of early round spermatids (rsd). Note prominent multinucleated spermatid (mrsd). Primary magnification, x 40.

Meiotic arrest is regularly found in patients showing non obstructive azoospermia being considered as idiopathic, because no genetic or other origin can be detected. There are numerous studies showing lack of expression of several genes in meiotic maturation arrest compared to normal spermatogenesis. A major subgroup of patients lacks BOULE protein expression in primary spermatocytes, which is key factor of meiosis (38). The defect seems to be due to factor(s) upstream of BOULE being involved in the transcription and/or translation of BOULE. Heat shock protein levels are low or absent, such as heat shock transcription factor, Y chromosome (HSFY) (39) or HSPA2 that is involved in DNA mismatch repair (MMR) (40). SYPC3, a gene responsible for the synaptonemal complex is also involved in MMR and was found to be reduced. There is increasing evidence that alterations of the SYPC3 gene are involved in spermatocyte maturation arrest. Although expression of SYCP3 mRNA is found in patients showing normal spermatogenesis and spermatocyte maturation arrest, the lack of expression in men with spermatogonial arrest, Sertoli Cell Only syndrome, and testicular atrophy suggests negative effect on spermatogenesis and male fertility (41). However, data concerning the involvement of SYCP3 mutations related to spermatocyte arrest are inconsistent. A mutation analysis of the SYCP3 gene for 58 patients revealed only polymorphisms (42). Miyamoto et al. found a 1 bp deletion (643delA) resulting in a truncation of the C-terminal region of the SYCP3 protein in two of 19 azoospermic men with maturation arrest versus 75 patients showing normal spermatogenesis (43). Recently Stouffs et al. detected one change present in an evolutionary important functional domain of the SYCP3 gene in only one male patient that was absent in more than 200 controls (44).

MicroRNA-383 was shown to be down-regulated in maturation arrest (45). It was associated with a hyperactive proliferation of germ cells in patients with mixed patterns of maturation arrest, indicating that miR-383 functions as a negative regulator of proliferation. The authors concluded that abnormal testicular miR-383 expression may potentiate the connections between male infertility and testicular germ cell tumor (46). There is a possible feedback loop between the fragile X mental retardation protein (FMRP) and miRNA-383, and FMRP acts as negative regulator for miRNA-383 functions, a loop that seems to be disturbed in maturation arrest (47).

Increased apoptotic index associated with spermatocyte maturation arrest was reported (29–31), data that correspond to the lack of expression of survivin, an inhibitor of apoptosis (48). These data correspond to the reduction of cyclin A, required for both the mitotic and meiotic divisions, in meiotic arrest (49).

In tubules showing meiotic arrest, there is also disturbance of the expression pattern of genes that are required for spermiogenesis. For example, BET (bromodomain and extra terminal) genes encode for transcriptional regulators and for histone-interacting chromatin remodelers. BRDT (bromodine testis specific), a key molecule participating in chromatin remodeling, is required for creation and/or maintenance of the chromocenter in round spermatids, a structure that forms just after completion of meiosis (for review see (50). The BRDT protein is localized in the nuclei of spermatocytes, spermatids, and ejaculated spermatozoa, and transcription is almost zero in primary spermatocytes of testes showing meiotic arrest (51). These data indicate that genes being important for postmeiotic spermiogenesis are already disturbed in the premeiotic stage.

In some patients with predominant round spermatid maturation arrest, the expression of cAMP Responsive Element Modulator (CREM) is significantly reduced or undetectable (52). Most recently, different expression of chromatin remodeling factors between normal spermatogenesis and round spermatid maturation arrest were found and suggest that impaired epigenetic information and aberrant transcription represents one reason for spermatid maturation arrest (53). Studies of the numerous mouse knock out models that display a 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 (54; 55).

Spermiogenesis is a complex process with numerous different factors being involved. Thus it should be noticed that many factors are described and will be found to be related or responsible for spermatid maturation arrest, such as Krüppel-like factor 4 (KLF4), a transcription factor which is involved in many cellular and developmental processes including terminal differentiation of cells and carcinogenesis. A significant altered subcellular localization in arrested spermatids gives a first hint at a role for KLF4 during spermiogenesis (56).

Data concerning the topic of possible epigenetic alterations related to spermatogenic defects are rare. Khazamipour et al. analyzed the methylation status in the specific CpG island of the promoter region of MTHFR (Methylenetetrahydrofolate reductase) and found a significant hyper-methylation in 53% of the patients showing NOA compared to 0% of patients with obstructive azoospermia and normal spermatogenesis, indicating that hyper-methylation is specific and not due to a general methylation defect (57). Authors suggest that epigenetic silencing of MTHFR may be involved in azoospermic infertility. A similar study analyzing the CpG island containing tissue specific differentially methylated regions (TDMRs) in the VASA gene revealed significantly higher methylation in maturation arrest compared to normal spermatogenesis (58). Hyper-methylation associated silencing of PIWIL2 and TDRD1 was reported by Heyn et al. in human infertile patients showing maturation arrest (59).

Adiga et al. evaluated the expression pattern of a DNA methyltransferase (DNMT3B) which is important for germ cell methylation (60). Although they found a reduced number of DNMT3B positive primary spermatocytes in the case of bilateral maturation arrest, the few mature spermatids did not reveal any alterations of global methylation status.

Additionally, there may also be extratesticular factors such as long standing ischemia due to malformation of valves in spermatic veins responsible for maturation arrest (61). Secondary factors for spermatogenetic arrest are toxic substances (radiotherapy, chemotherapy, antibiotics), heat or general diseases (liver or kidney insufficiency, sickle cell anaemia) (62).

Testicular volume, FSH and inhibin B may be in their respective normal range, but may also be elevated or decreased. When these clinical parameters are normal, the differential diagnosis includes obstructive and non-obstructive azoospermia and this distinction made by diagnostic biopsy.

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 likely that many genetic factors exist but have not yet been identified.

Complete arrest of spermatogenesis results in azoospermia. To date, there is no known therapy for uniform spermatogenic arrest (63).

HYPOSPERMATOGENESIS

The histological phenotype “hypospermatogenesis” shows complete spermatogenesis, but the number of elongating or elongated spermatids is moderately or severely reduced and the composition of the seminiferous epithelium is often incomplete because of missing generations of germ cells.

There are numerous reports showing functional impairment or alterations in seminiferous tubules showing hypospermatogenesis. Hypospermatogenesis is often associated with multinucleated

spermatids indicating failure in spermiogenesis, or with so-called “megalospermatocytes” that are the morphological representation of missing synaptonemal complexes during meiotic prophase (64; 65). Whereas mitotic activity of spermatogonia is reduced (66), the apoptotic index indicating increased germ cell degeneration is elevated as shown by caspase immunohistochemistry (31) or TUNEL analysis (30). Both are also true in the case of maturation arrest at different levels of germ cell development.

Concentric spherical concrements deriving from the basal lamina are often found, when ultrasonographic examination of the testis reveals “microlithiasis”. These concrements may be associated with carcinoma in situ (syn: testicular intraepithelial neoplasia: TIN).

During spermiogenesis, protamine mRNA, being associated with the prognosis of successful ICSI therapy, is reduced in early round spermatids (67; 68). The histone to protamine transition during spermiogenesis is due the transcription factor CREM (cAMP responsive element modulator) and CREM activators. There are different isoforms functioning as activators and repressors and the expression pattern is related to impaired spermatogenesis (69–71).

Sertoli cell function is impaired, which has been described by Bruning et al. (72) by three dimensional reconstruction indicating functional dedifferentiation. This phenomenon, found to be associated with numerous aspects of Sertoli cell function, was later reviewed by Sharpe et al. (73). Most recently, Fietz et al. (74) could show a reduced mRNA expression of the androgen binding protein by quantitative RT-PCR. Huthaniemi et al. (75) found increased testosterone levels associated with androgen receptor CAG repeat length and because of a constant testosterone to estrogen ratio, authors suggested increased estrogen levels to be responsible for impaired spermatogenesis. Contrary data were reported by Nenonen et al. (76) who found a non-linear association between androgen receptor CAG repeat length and risk of male subfertility. This meta-analysis including almost 4000 patients revealed that androgen receptors with both either short or long repeats displayed lower activity than the receptors with repeats of median length. On a cellular level, Fietz et al. (74) analyzed androgen receptor mRNA of Sertoli cell populations associated with defined spermatogenic impairment using laser assisted cell picking and did not find any correlation of CAG repeat length to testicular histology or AR expression, suggesting factors other than CAG repeat to be responsible for severe spermatogenic impairment including mixed atrophy. This was also found by Hadjkacem-Loukil et al. (77) in a cohort of Tunesian azoospermic men showing Sertoli Cell Only syndrome or maturation arrest.

The lamina propria looks mostly unaffected in routine histological sections. However, functional defects resulting in a loss of contractility i.e. such as myosin heavy chain (MHY11) (78) or smooth muscle actin (79) were associated with hypospermatogenesis or mixed atrophy.

Functional dedifferentiation was found in Leydig cell hyperplasia and adenoma indicated by downregulation of the Leydig cell specific relaxin-like factor using in situ hybridysation and immunohistochemistry (80).

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 germ cell aplasia (81). Several studies have demonstrated that inhibin B is a more sensitive and specific endocrine marker of hypospermatogenesis (82; 83). However, even the combined measurement of inhibin B and FSH provides no certainty concerning the presence or absence of sperm in multiple testicular biopsies (84; 85).

Mixed Atrophy

In most oligozoo- or azoospermic patients, testicular biopsy reveals a pattern of different spermatogenic defects in adjacent tubules: “mixed atrophy” being first described by Sigg (86): the simultaneous occurrence of seminiferous tubules includes SCO tubules or even only lamina propria (tubular shadows). This requires a detailed score-count analysis (35; 37). Additionally, functional mRNA or protein analysis of gene expression pattern described above can help to optimize the diagnosis of the underlying defects.

From a practical clinical perspective, the differentiation is important as patients with hypospermatogenesis or mixed atrophy may have azoospermia or varying degrees of oligoasthenoteratozoospermia, and sperm may be retrieved from testicular biopsies (TESE) (35). Pregnancies can be achieved with sperm retrieved by TESE 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 germ cell aplasia. Clinical studies performed so far have demonstrated some increase in sperm concentration in the ejaculate and improvement of pregnancy rate (87; 88).

NUMERICAL CHROMOSOME ABERRATIONS

Klinefelter Syndrome

Harry Klinefelter first described this syndrome in 1942 as a clinical condition with small testes, azoospermia, gynecomastia and an elevated serum FSH (89). Only in 1959 was the chromosomal basis of the disorder elucidated as the chromosomal constitution with a supernumerary X-chromosome. Subsequently, the diagnosis of Klinefelter syndrome is made by chromosome analysis demonstrating the 47,XXY karyotype or one of its rarer variants.

The prevalence of Klinefelter syndrome is approximately 1 in 1,000 to 1 in 500 males (90). It is the most frequent form of primary testicular dysfunction affecting spermatogenesis as well as hormone production and is found in about 3% of unselected infertile men and >10% of men presenting with azoospermia (91; 92). It appears that at least half of the cases remain undiagnosed and untreated throughout life (90).

A non-mosaic 47,XXY karyotype is found in 80 - 90 percent of Klinefelter patients and mosaicism is seen in another 5 - 10 percent. The 47,XXY/46,XY mosaicism is most common. The 48,XXXY, 48,XXYY and 49,XXXXY karyotypes constitute 4 - 5 percent of all Klinefelter syndrome karyotypes, structurally abnormal extra X chromosomes are found in less than one percent of patients. Apart from karyotype analysis, molecular genetics methods can be used to quantify the number of X chromosomes, for example by quantitative PCR analysis of the androgen receptor gene located on the X chromosome (93).

The numerical aberration in non-mosaic 47,XXY is derived with equal likelihood from maternal or paternal meiotic error (94; 95). Most cases are caused by meiosis without X/Y or X/X recombination. Advanced maternal age seems to be a risk factor (90). 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.

Patients with Klinefelter syndrome are usually inconspicuous until puberty. Interestingly the velocity of height gain can be increased in the pre-pubertal years. Men with Klinefelter syndrome tend to be tall (mean adult height is about the 80th percentile for the population) and to have relatively long legs compared to their overall height. Previously, the tall stature in KS was mainly thought to be a consequence of the hypogonadism, i.e. lower testosterone/estradiol levels not stopping long-bone growth by inducing epiphyseal growth plate fusion. However, more recent data comparing gonosomal aneuploidies support that increased body height is caused by excessive expression of growth-related genes. In this respect, the *SHOX*-gene is the leading candidate as it is located in the pseudoautosomal region and therefore present in three copies in Klinefelter men (96).

In most patients, early stages of puberty proceed normally. Post-pubertally the syndrome is characterized by small testes with firm consistency remaining in the range of 1 - 4 ml. Most patients with Klinefelter syndrome are infertile because of azoospermia. Testicular histopathology in adult men with Klinefelter syndrome displays various patterns. Classically, germ cell aplasia, total tubular atrophy or hyalinizing fibrosis and relative hyperplasia of Leydig cells are found. However, in some adult Klinefelter patients, foci of spermatogenesis up to the stage of mature testicular sperm can be detected ((97), and see below).

The degree of virilization varies widely. In early puberty, LH and FSH increase while serum levels of testosterone plateaus at or just below the lower limit of the normal range. After the age of 25, about 80% of patients have reduced serum testosterone levels and complain of decreasing libido and potency. On average, serum estradiol levels are high normal or may exceed the normal range. LH and especially FSH levels are exceedingly high, serum levels of inhibin B are very low or undetectable (98; 99).

During puberty, bilateral painless gynecomastia of varying degrees develops in about half of the patients. In a large Danish study covering 696 men with Klinefelter syndrome, no evidence for a substantial increase in the overall cancer rate was found (100). The risk of developing mammary carcinoma may be increased relative to normal men but remains a rare occurrence and routine surveillance is not recommended (100; 101). A significantly increased risk was found for the rare mediastinal malignant germ cell tumors, which occur preferentially at the age of 14 to 29 years (100).

The intelligence of Klinefelter patients is very variable. The group difference between boys with Klinefelter syndrome and controls amounts to 11 points in full scale IQ (92 versus 103), and deficits are observed primarily in verbal and cognitive abilities (102). Some of the young patients attract attention because of learning difficulties and school problems. They may fail to reach the level of achievement or professional expectations of their families (103; 104). Compared with their classmates, certain abnormal physical and psychological characteristics of the patients become obvious and they may become socially alienated. Higher-grade aneuploidy of the sex-chromosomes (48,XXXY, 48,XXYY and 49,XXXXY) is associated with mild to severe mental retardation while Klinefelter patients with chromosomal mosaicism (47,XXY/46,XY) may show very few clinical symptoms.

In general, the variability of the clinical features in patients with Klinefelter syndrome is related to the degree of androgenisation, which, in turn, partly depends on the pattern of inactivation of one copy of the androgen receptor gene. In particular, a significant genotype-phenotype association exists in Klinefelter patients and androgen effects on appearance and social characteristics are modulated by the androgen receptor CAGn polymorphism (105; 106).

Regarding infertility treatment, it should be noted that in rare cases sperm could be found in the ejaculate and, exceptionally, spontaneous paternity has been described (107). The rate of diploidy of sperm as well as disomy for gonosomes and autosomes has been reported to be increased in patients with Klinefelter syndrome, however, the majority of sperm appear to be normal (108–111). Almost two decades of experience with TESE/ICSI in patients with Klinefelter syndrome demonstrates that testicular sperm can be recovered in about 50% of the patients (112–115). Increasing age may be a negative predictive factor for successful TESE and some advocate to offer TESE and cryopreservation of tissue/spermatozoa already to teenaged patients. To what extent other factors such as previous testosterone treatment influence the chances of successful TESE remains under debate, as does the suggested treatment with drugs increasing FSH prior to TESE (116). So far, over 170 babies were born using testicular sperm for ISCI, all showing normal karyotype, although aneuploidies can be occasionally found by preimplantation or prenatal diagnosis (117). However, since the birth of normal children conceived by assisted reproductive techniques seems to be the rule (115), preimplantation diagnosis is not per se indicated. Based on indirect clues, it was postulated that 47,XXY spermatogonia are able to complete meiosis (118). However, Sciurano et al. nicely showed by fluorescence in situ hybridization (FISH) in testicular tissue of Klinefelter patients that all meiotic spermatocytes were euploid 46,XY(119). Fittingly, the common birth of children with normal karyotype suggests that the few sperm which can be found in patients with Klinefelter syndrome derive from the clonal expansion of spermatogonia with normal karyotype.

When testosterone serum levels are reduced, substitution with testosterone is necessary. To avoid symptoms of androgen deficiency, hormone replacement therapy should be initiated as early as needed. In particular, Nielsen et al. (120) showed that early testosterone replacement not only relieves biological symptoms such as anemia, osteoporosis, muscular weakness and impotence, but also leads to better social adjustment and integration. However, concurrent testosterone treatment severely reduces the chances of successful TESE and, therefore, the option of TESE should be considered before starting the first testosterone substitution and otherwise treatment should be stopped before the biopsy. Testosterone 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.

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 fluorescence in situ hybridization or molecular methods it has been demonstrated that about 80% of XX-males have Y chromosomal material translocated onto the tip of one X chromosome (121). Translocation of a DNA-segment which contains the testis-determining gene (*SRY* = Sex Determining Region Y) from the Y to the X chromosome takes place during paternal meiosis (122). The presence of the gene is sufficient to cause the initially indifferent gonad to develop into a testis. The breakpoints and consecutively the size and content of the translocation seem to influence the severity of the phenotype (123).

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, which is in line with the recent view that the number of sex-chromosomes (most likely copies of the *SHOX*-gene) largely determines final height (96). The

incidence of maldescended testes is significantly higher than that in Klinefelter patients and controls (124). The testes are small (1 - 4 ml) and firm, and endocrine changes of primary testicular failure with decreased serum testosterone and elevated estrogen and gonadotropin levels are observed. 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 germ cells.

In *SRY*-negative XX-males (about 20% of XX-males), mutations in *SOX9, RSPO1* or other candidate genes may be responsible for the sex reversal, but these are very rare and the mechanism underlying the majority of cases currently remains unclear (125). *SRY*-negative XX-males are generally less virilized than *SRY*-positive men and may show additional malformations of the genital organs such as maldescended testes, bifid scrotum or hypospadias (126).

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.

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 non-mosaic chromosomal aneuploidy is caused by non-disjunction 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 1:1,000.

Men with 47,XYY-syndrome have serum levels of testosterone and gonadotropins, as well as testicular volumes, comparable to those of normal healthy men. Most men with 47,XYY-syndrome have normal fertility. Onset of puberty seems to be delayed by 6 months, adult height is 7 cm in excess of the male population mean. The intelligence quotient lies within the normal range, but men score an average of ten points less than age-matched peers. Behavioral problems are more common in 47,XYY males, however, a history of violent behavior is exceptional (127; 128).

Most 47,XYY-men do not need any specific therapy. Men who achieve fatherhood can expect chromosomally normal offspring probably with the same likelihood as normal men. Nevertheless, to be safe, prenatal diagnosis can be offered.

STRUCTURAL CHROMOSOME ABERRATIONS

Structural chromosome abnormalities encompass alterations of chromosome structure that are detectable through light-microscopic examination of banded metaphase preparations as well as smaller, sub-microscopic deletions and duplications that are only detectable with molecular genetics (e.g. array Comparative Genomic Hybridization, aCGH). Structural rearrangements such as Robertsonian translocations, that also imply a change in chromosome number, are also regarded as structural abnormalities.

Structural anomalies of the autosomes are distinguished from anomalies of the sex chromosomes (gonosomes). Especially reciprocal and Robertsonian translocations, inversions, marker chromosomes, X and Y isochromosomes, and Y chromosomal deletions are of practical importance for andrology. When evaluating a structural chromosomal anomaly for clinical purposes, the

distinction between balanced and unbalanced structural aberrations is pivotal. The former are characterized by a deviation from normal chromosome structure but without a net loss or gain of genetic material. If no important gene is disrupted at the breakpoints, balanced structural aberrations exert no negative effect on general health but may cause spermatogenic failure (oligo- or azoospermia) and independent of that, an increase in the risk for unbalanced karyotypes in the offspring (91; 129; 130).

In unbalanced structural chromosomal abnormalities, genetic material is either missing or there is an overall net excess of material in the cell. Unbalanced chromosomal aberrations may be incompatible with life and lead to abortion or cause a broad spectrum of disease. Exceptions are deletions of the Y chromosome that may limit reproductive functions selectively, and are therefore of importance in reproductive medicine (see below).

The majority of male individuals carrying structural aberrations is probably fertile and need no specific therapy. Conversely, men with impaired spermatogenesis show an increased prevalence of structural chromosomal abnormalities (129; 91; 92). Infertile patients with structural chromosomal aberrations may conceive naturally while more severe cases may require 'symptomatic' treatment modalities such as intracytoplasmic sperm injection, however, success rates may be lower than in couples with normal karyotypes (131). It should also be considered that unbalanced karyotypes of the embryo may result from balanced parental chromosomal anomalies (132). For any carrier of a structural chromosome abnormality who considers fatherhood by any means, genetic counselling is strongly recommended, and it should be obligatory prior to any infertility treatment (133; 134). It should be mentioned that in many countries karyotyping of men with idiopathic infertility and decreased sperm concentration is recommended prior to ICSI therapy although an evidence based screening threshold does not exist (135). The risk of spontaneous pregnancy loss, congenital malformations regularly associated with developmental delay as a result of an unbalanced karyotype in the offspring, options of prenatal and preimplantation genetic diagnosis, and - for certain aberrations - the possibility that other family members are also affected should be discussed with the patient.

Structural Aberrations 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 (135).

Translocations and other structural chromosomal aberrations can be either a *de novo* occurrence in the subject or inherited. Therefore, testing in family members should be encouraged, as the presence of a chromosomal aberration is regularly associated with a higher rate of abortion and the risk for the birth of a severely handicapped child.

Structural Aberrations of Sex Chromosomes

An 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 (136). The *SRY* gene is localized on the short arm of the Y chromosome and it influences differentiation of the embryonic gonad into the testicular pathway. The long arm of the Y chromosome contains areas responsible for establishing regular spermatogenesis.

When speaking of deletions of the Y chromosome, those of the short and the long arm must be distinguished (137). Short arm deletions of the Y chromosome that encompass the sex determining *SRY* gene result in sex reversal. Clinically, affected subjects 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 harbors loci essential for spermatogenesis (136).

In addition to deletions, a series of further structural anomalies of the Y chromosome are known. Pericentric inversions generally remain 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 ambiguous. Patients with a male phenotype are usually infertile. These patients have an increased risk of developing testicular tumors (see Endotext.com, Endocrinology of Male Reproduction, Chapter 13: Testicular cancer pathogenesis, diagnosis and endocrine aspects). Reciprocal translocations between the Y chromosome and one of the autosomes are rare. In most cases, spermatogenesis is severely disturbed. However, several men with these aberrations have been reported as 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.

The X chromosome contains numerous genes essential for survival. Every major deletion of this chromosome has a lethal or severe 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 MICRODELETIONS

The human Y chromosome is not only the dominant sex determinator, but plays an essential role in the genetic regulation of spermatogenesis (138). The long arm of the Y chromosome contains three partially overlapping but discrete regions that are essential for normal spermatogenesis (136; 139). The loss of one of these regions, designated as AZF (azoospermia factor)a, AZFb (P5/proximal P1), AZFc (b2/b4), and AZFbc (with two variants differing in the proximal breakpoint: P5/distal P1 and P4/distal P1) can lead to infertility (136). The deleted regions are usually of submicroscopic dimensions and are known as Y chromosomal microdeletions. Their prevalence in azoospermic men lies between 5 - 10% and between 2 - 5% in cases of severe oligozoospermia (140). Clearly, the frequency of Y microdeletions is related to the criteria by which men have been selected (141; 142), whereas ethnic differences might exist as well (143). Deletions of the AZFc region represent about 80% of all AZF deletions (143). The type and mechanism of deletions have been recently clarified and result from homologous recombination between retroviral or palindromic sequences

(144). The AZFc region includes 12 genes and transcription units, each present in a variable number of copies making a total of 32 copies (145). The classical complete deletion of AZFc (b2/b4 deletion), removes 3.5 Mb, corresponding to 21 copies of genes and transcription units (146). Even more gene copies are removed by more extensive deletions (7.7 Mb and 42 copies removed in P5/distal P1 deletions; 7.0 Mb and 38 copies removed P4/distal P1 deletions) (147). It remains unclear if any of the genes of the respective regions are indeed pathologically relevant.

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 syndrome (SCO) to spermatogenic arrest or hypospermatogenesis with qualitatively intact but quantitatively severely reduced spermatogenesis (148; 143). In azoospermic men, the presence of a complete deletion of AZFa seems to be associated with uniform germ cell aplasia (complete SCO), while a histological picture of SCO or spermatogenic arrest seems common in men carrying complete AZFb or AZFbc deletions. However, in exceptional cases, complete AZFb-deletions seem compatible with finding, albeit very few, spermatozoa (149; 150). Overall, the chances for successful sperm retrieval in carriers of complete AZFa as well as AZFb and AZFbc deletions has still to be considered virtually zero. On the other hand, men carrying complete AZFc deletions have severe oligozoospermia in about 50% of cases and in azoospermic carriers, successful TESE seems possible in about half of them (148; 151). No clinical parameter can help distinguishing patients with microdeletions of the Y chromosome from infertile men without microdeletion and, therefore, screening of all men with severe oligo- or azoospermia and without other causes is indicated (143; 135). It should be noted that Y chromosome microdeletions have also been described in proven fertile men (152).

A positive result of the analysis, which should be carried out according to the standard recommended by the current guidelines (153), provides a causal explanation for the patient's disturbed spermatogenesis. Beyond this, the test also has prognostic value, as TESE is possible in about 50% of men with AZFc deletion and every son of such a patient will carry the paternal Y chromosomal microdeletion and thereby inherit disturbed fertility (154). Hence, genetic counseling is indicated for all carriers of Y chromosomal microdeletions (133; 148).

Smaller deletions removing only part of the AZFc region have been identified as a polymorphism significantly associated with infertility, especially oligozoospermia (145). These so-called gr/gr deletions arise by the same mechanism (homologous recombination) and have been extensively studied in large groups of men in different countries. Overall, they are found in about 6.8% of infertile men but also in 3.9% of the controls and four meta-analyses have reported significant Odds Ratios, reporting on average 2-2.5 fold increased risks of reduced sperm output/infertility (55; 155–157). Although they represent a significant risk factor for male infertility, they should be regarded as a polymorphism and for the time being this type of diagnostics offers no advantage in male infertility workup. Concerns have been raised that a gr/gr (partial AZFc) deletion may expand to a complete AZFc deletion in the next generation and gr/gr deletions have also been reported as risk factor for testicular cancer (158; 159). Currently, however, no general agreement to advise routine testing has been reached (55; 157; 160; 153).

OUTLOOK: NEW TECHNOLOGIES, NOVEL GENETIC CAUSES

For many years, single candidate genes have been evaluated - usually by genotyping single nucleotide polymorphisms or sequencing - with the goal of identifying causal mutations for spermatogenic failure. Most of these approaches were, however, not successful most probably because 1) “male infertility” as well as “spermatogenic failure” are highly genetically heterogeneous

and 2) selection of patient groups is often not stringent. Conversely, with the advances in genetic technologies, namely array-Comparative Genomic Hybridization (array-CGH) and whole-exome or even -genome sequencing, it is now possible to perform unbiased genome-wide analyses. These novel methodologies easily outperform the previous candidate gene approach which is illustrated by an increasing number of recent publications of so-called Copy Number Variations (CNVs), larger DNA regions that may be duplicated or deleted, as well as single genes causing spermatogenic failure. Examples are studies presenting CNVs on the autosomes as well as sex-chromosomes that are associated with azoo- or severe oligozoospermia (34; 161–163) as well as genes that are frequently mutated in specific phenotypes like meiotic arrest (163). In the near future, these novel technologies will help to greatly increase the fraction of men with a clear genetic diagnosis.

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