Developmental and genetic disorders in spermatogenesis

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The most common cause of male infertility is idiopathic. Fresh insights based on genetic and molecular analysis of the human genome permit classification of formerly unexplained disorders in spermatogenesis. In this article, we review new procedures that expand diagnostic and therapeutic approaches to male infertility. Recombinant DNA technology makes it possible to detect specific chromosomal and/or genetic defects among infertile patients. The identification of genes linked to disorders in spermatogenesis and male sexual differentiation has increased exponentially in the past decade. Genetic defects leading to male factor infertility can now be explained at the molecular level, even though the germ cell profile of infertile patients is too variable to permit classification of the clinical phenotype. Increasing knowledge of genes that direct spermatogenesis provides important new information about the molecular and cellular events involved in human spermatogenesis. Molecular analysis of chromosomes and/or genes of infertile patients offers unique opportunities to uncover the etiology of genetic disorders in spermatogenesis. Increasing numbers of cases, previously classified as idiopathic, can now be diagnosed to facilitate the treatment of infertile men. Advanced knowledge also poses ethical dilemmas, since children conceived with assisted reproductive technologies such as intracytoplasmic sperm injection (ICSI) are at risk for congenital abnormalities, unbalanced complements of chromosomes and male infertility.

Key words: chromosomal aberrations/infertility/male sexual differentiation/microdeletions/testis

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Introduction

Analysis of infertility cases worldwide indicates that the largest percentage of these patients experience idiopathic infertility (Figure 1), typified by severe oligozoospermia or azoospernia (Fisch and Lipshultz, 1992; Martin-DuPan et al., 1997; Nieschlag, 1997; Sigmann et al., 1997; Zargar et al., 1997). Investigation of chromosomes from patients with azoospermia or oligozoospermia offers fresh insights that begin to explain some of the causes of idiopathic infertility at the level of specific genes, particularly those associated with the X and Y chromosomes (Jaffe and Oates, 1997). Chromosomal defects formerly identified by cytological techniques can now be explained on the basis of substitutions in individual base pairs within a gene (Tuerlings et al., 1997; Simoni et al., 1998).

Genes on sex chromosomes have been implicated in a growing number of spermatogenetic disorders because recessive mutations and deletions on the X and Y chromosomes exert a direct impact on phenotypic sex. The absence of effective crossing-over in meiosis caused by negligible homology of large parts of the sex chromosomes makes them susceptible to recessive defects (Handel and Hunt, 1992). Recombinant DNA techniques provide the potential for explaining as much as 10% of the idiopathic forms of infertility that have heretofore gone undiagnosed (Simoni et al., 1998; Tuerlings et al., 1998b). Adoption of new molecular procedures for detecting...
aberrations in chromosomes or defects in genes has had profound investigative, clinical and ethical implications.

From an investigative standpoint, the use of recombinant DNA techniques provides new insights into the mechanisms directing germ cell maturation at all stages of spermatogenesis. At the clinical level, many new diagnostic approaches are available, and these enlarge the therapeutic options for managing clinical disorders in spermatogenesis that were formerly untreatable. New diagnostic protocols also expand the opportunities to make patients aware of their disorder and facilitate prediction of the risks of transmitting a genetic disorder to offspring. From an ethical perspective, prudence and caution should be exercised in recommending assisted reproductive protocols to infertile couples. Children born following the use of intracytoplasmic sperm injection (ICSI) are more likely to experience developmental abnormalities and male infertility than those conceived naturally or with in-vitro fertilization (IVF) (Andrews et al., 1998; Bonduelle et al., 1998a,b; Bowen et al., 1998).

In this article, we review the chromosomal, genetic and clinical aspects of disorders in spermatogenesis, with a focus on identifying genes responsible for disorders in germ cell differentiation. Attention then shifts to genetic disorders interfering with testicular development and male sexual differentiation. New diagnostic and therapeutic approaches are emphasized that will assist physicians with diagnosis and treatment of male infertility.

**Therapeutic approaches to disorders in spermatogenesis**

Primary endocrine failure involving either gonadotrophin-releasing hormone (GnRH) or gonadotrophin deficiency is associated with severe disorders in spermatogenesis (Behre et al., 1997). Such disorders can be treated successfully by replacement therapy with either GnRH or a combination of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Behre et al., 1997).

Other disorders in spermatogenesis fail to respond to endocrine therapy and require interventional approaches such as ICSI (Palermo et al., 1992) when only a few spermatozoa are available for recovery from an ejaculate (Harari et al., 1995). Patients with azoospermia can be assisted if spermatozoa are recoverable from the epididymal duct following epididymal aspiration or can be isolated from the seminiferous epithelium after testicular biopsy (Silber et al., 1995). Round spermatids have been used successfully with ICSI when spermatogenesis is arrested at the late spermatid stage, but optimal clinical success is achieved with motile spermatozoa (Fishel et al., 1995; Tesarik et al., 1995; Vanderzwalmen et al., 1995). Fertilization rates of 35% and pregnancy rates of ~25% represent current standards for ICSI when spermatozoa are recovered from azoospermic patients (Kahraman et al., 1996). Genetic risks and developmental delays following use of the ICSI procedure should be considered, since, according to some studies, the probability that offspring may be affected adversely is significantly greater than after IVF or natural fertilization (Andrews et al., 1998; Bonduelle et al., 1998a,c; Bowen et al., 1998).

No known therapeutic approaches are available to men when spermatogenesis is halted prior to the completion of meiosis. The fraction of patients who lack post-meiotic germ cells is relatively small (Silber et al., 1995), but the most profound disorders in spermatogenesis are prevalent in these patients. Patients lacking post-meiotic germ cells may be assisted in the future, but as yet no known treatment can prompt germ cells to complete meiosis and proceed with spermiogenesis.

**Chromosomal aberrations**

Chromosomal aberrations occur as two types: numerical and structural. Numerical aberrations arise from a missing or accessory chromosome due to meiotic non-disjunction in either maternal or paternal germ cells. Accessory chromosomes, such as trisomies and other polyploidies, may involve either an autosomal or sex chromosome or both. Numerical abnormalities in sex chromosomes typically occur in newborns without apparent defects in the external genitalia. Somatic structures are usually normal, but profound deviations can occur. The severity of defects appears to depend on the grade of sex chromosome polyploidy, and such defects are more likely in patients...
with cell lines containing multiple X chromosomes or an X0 cell line. Trisomies, involving autosomes, usually are associated with severe somatic defects, including mental retardation, abnormal stature or heart failure.

Structural aberrations involve the loss or duplication of genetic information, including the translocation of genetic information from one chromosome to another without loss in a numerically normal complement of chromosomes. Gross structural aberrations in a chromosome are detectable by microscopic inspection of the karyotype, while the microdeletion of a gene or a single base pair mutation can only be detected by analysis of DNA from a patient of interest. Point mutations represent the smallest structural aberration in a single base pair within a gene. Aberrations involving structural rearrangements in a chromosome are known to cause errors in promoter or repressor sequences of multiple genes, leading to profound disturbances in spermatogenesis (Nieschlag et al., 1997; Tuerlings et al., 1998b). Structural aberrations explain a wide range of phenotypic abnormalities, including a host of defects in spermatogenesis that are impossible to classify because hundreds of genes are required for the production of spermatozoa (Engel et al., 1996).

**Classification of structural aberrations in chromosomes**

Deletion refers to the loss of genetic information. Deletions occur interstitially or terminally when one arm of a chromosome is affected. Deletions range from the microdeletion of a single gene to macroscopic losses in parts of a chromosome (Mak and Jarvi, 1996).

Peri- and paracentric inversion involves the rearrangement of genetic information without the loss of chromosomal material. Defects attributed to gene reorganization depend upon the localization of the breakpoints and the number and function of affected genes (Meschede et al., 1994).

Translocation involves the transfer of a part of a chromosome to another arm of the same or to another chromosome. Translocations can be balanced or unbalanced. Unbalanced translocations imply that the portion of the transferred chromosome is lost or duplicated, with the retention of all information in the balanced carrier (Xing and Lawrence, 1993; Chandley et al., 1975; Mak and Jarvi, 1996).

Robertsonian translocation refers to a special condition where fusion occurs between two acrocentric chromosomes with the loss of genetic information from the short arms of participating chromosomes (Mak and Jarvi, 1996).

Base pair mutations can occur in all genes. Substitution or deletion of a single base sequence in a strand of DNA can have a profound impact on gene function (Quinton et al., 1996; Cameron and Sinclair, 1997). The mutation may produce a failure in transcription or a frameshift resulting in the complete loss of an encoded protein. Recessive mutations have no impact on the affected individual but are inherited and transferred to future generations.

**Sex chromosomes**

**X-chromosome aberrations**

Infertility is caused by minor deletions or translocations of genes on the X chromosome (Meschede et al., 1998). The most profound defects in testicular development are caused by deletion of a major portion of the X chromosome. Such deletions are incompatible with the development of a male fetus, since the loss of one or more genes on the X chromosome in males is not compensated by the presence of genes from a second X chromosome, as is the case for females.

**Xp22 contiguous gene syndrome.** The Xp22 contiguous gene syndrome is a rare disorder and treatment of infertility is not a clinical objective due to multiple defects such as anosmia, mental retardation and short stature (Kletter et al., 1991). De-novo deletion of Xp22-pter from the X chromosome (Figure 2) affects several genes, including those responsible for glycerol kinase deficiency (GKD), adrenal hypoplasia congenita (DAX-1), Duchenne type muscular dystrophy (DMD) (Kletter et al., 1991) and Kallmann’s syndrome (KAL-1) (Figure 2). KAL-1 is deleted in ~50% of the patients with the Xp22 contiguous gene syndrome, causing a primary deficiency in GnRH production. Patients lacking GnRH are hypogonadal and fail to produce spermatozoa.

**Translocations of X-chromosome genes to autosomes and the Y chromosome.** Translocation of genes on the X chromosome to either autosomes or the Y chromosome is not a common cause of male infertility (Chandley et al., 1975; Gabriel-Robez et al., 1990; Yoshida et al., 1997). X chromosome translocations are hereditary and the impact on offspring is unpredictable because multiple genes are affected.

Balanced translocations of genes on the X chromosome to either autosomes or the Y chromosome interfere with male sexual differentiation or spermatogenesis (Chandley et al., 1998). Such translocations typically occur at breakpoints in the pseudoautosomal boundary region of the X chromosome (Xp22-pter) and in the Yq11 region of the Y chromosome. The Yq11 region contains several genes located in the AZF (azoospermia factor) region (Figure 2) that regulate transcription and are required for spermatogenesis (Vogt, 1998). No genes from the pseudoautosomal boundary region of the X chromosome have been linked to spermatogenic arrest (Gabriel-Robez et al., 1990).

Normal spermatogenesis depends on X chromosome inactivation, a process directed by an X-linked gene during the spermatocyte stage of germ cell development. The pairing and recombination of the X and Y chromosomes, followed by X inactivation, is limited to the pseudoautosomal regions and occurs when both chromosomes are transcriptionally inactive, as indicated by chromatin formation (Handel and Hunt, 1992). X-chromosome inactivation functions to prevent re-
Disorders in spermatogenesis

Figure 2. Diagrammatic representation of sex chromosomes showing genes on the X and Y chromosomes that have been identified to be associated with disorders in either spermatogenesis or the differentiation of the male phenotype. Numbers in the centre of the Y chromosome designate intervals, and localizations are designated in the centre of the figure based on cytogenetic banding patterns. Genes affecting spermatogenesis are designated in bold type, other loci purported to control spermatogenesis are listed in brackets, and DNA markers for specific regions are shown in italicized type. Specific regions enclosed in brackets may be similar to those genes shown in bold type where they appear adjacent to each other. New information on genes mapping to the X and Y chromosomes can be obtained at http://www.nhgri.nih.gov/Data, a website maintained by the National Human Genome Research Institute.

combination between the X and the Y chromosomes during meiosis (Handel and Hunt, 1992; Jamieson et al., 1996). Translocations affecting the X chromosome appear to interfere with the process of X inactivation, resulting in meiotic arrest.

Translocations involving a portion of the X chromosome have a profound impact on spermatogenesis, as indicated by the failure of most spermatocytes to enter into meiosis (Jamieson et al., 1996). In certain cases, spermatogenesis can proceed to the formation of elongated spermatids, but the process is remarkably inefficient, as indicated by the presence of a few spermatozoa.

Table I. Disorders in spermatogenesis attributable to mutations and defects of the Y chromosome

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Disorder</th>
<th>Phenotype</th>
<th>Gonadal manifestation</th>
<th>Extra-gonadal manifestation</th>
<th>Spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yp11.3</td>
<td>SRY</td>
<td>XX-male</td>
<td>male</td>
<td>gonadal</td>
<td>sex</td>
<td>germ cells</td>
</tr>
<tr>
<td>Yq11.21</td>
<td>unknown</td>
<td>azoospermia</td>
<td>female</td>
<td>dysgenetic reversal</td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>AZF ‘a’</td>
<td>SMCY³</td>
<td>azoospermia</td>
<td>male</td>
<td>spermatogenesis</td>
<td>Sertoli only</td>
<td></td>
</tr>
<tr>
<td>AZF ‘b’</td>
<td>RBM1³</td>
<td>azoospermia</td>
<td>factor ‘b’</td>
<td>spermatogenic arrest</td>
<td>at level of spermatocytes or spermatids</td>
<td></td>
</tr>
<tr>
<td>Yq11.22–23</td>
<td>TSPY³</td>
<td>azoospermia</td>
<td>factor ‘c’</td>
<td>spermatogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZF ‘c’</td>
<td>DAZ³</td>
<td>azoospermia</td>
<td>male</td>
<td>defective</td>
<td>reduced spermatogenesis or arrest in spermatid development</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTY1+2³</td>
<td>azoospermia</td>
<td>spermatogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

³Tentative identification of a gene or multiple genes appear to be involved, but definitive identification and significance in the differentiation of germ cells remain to be established.
Infertile patients with X-linked translocations are candidates for assisted reproductive technologies, provided the translocations are not associated with other somatic defects. Patient selection should be based on the degree of spermatogenic failure; no treatment can be offered unless meiosis is completed and spermatogenesis proceeds to the formation of spermatids.

Sexual differentiation is affected by translocations in Xp21.2-pter (GDXY) (Figure 2). The Xp21.2-pter region appears to be crucial for the differentiation of the male phenotype, since duplication and translocation can give rise to a 46, XY dose-sensitive sex reversal with a feminine phenotype and gonadal dysgenesis (Pringle and Page, 1997).

DAX-1, a gene located at Xp21 (Figure 2), is a decisive X gene for sexual differentiation (Swain et al., 1998). Expression of DAX-1 in mice indicates it plays a critical role in early differentiation of adrenal and Sertoli cells in the genital ridge (Swain et al., 1998), but prolonged expression of DAX-1 interferes with testicular development. DAX-1 expression is down-regulated during the formation of the testis, while transcription persists during the development of the ovary in mice (Swain et al., 1998). Overexpression of DAX-1 in transgenic mice antagonizes the expression of SRY, a gene on the Y chromosome (Figure 2) required for the differentiation of male secondary sexual characteristics (Swain et al., 1998). Antagonism between DAX-1 and SRY explains X-linked forms of gonadal dysgenesis in humans (Swain et al., 1998).

Y-chromosome aberrations

Translocations involving the Y chromosome. Cytogenetic survey of infertile patients indicates that chromosomal abnormalities, including translocations, are 4–10 times more prevalent among infertile than fertile men (Chandley et al., 1975; Mak and Jarvi, 1996; Meschede et al., 1998). Translocation of a portion of the Y chromosome to autosomes typically occurs in chromosomes 1, 3 and 11 and impairs spermatogenesis (Sasagawa et al., 1993). Breakpoints in the Y chromosome frequently arise in the Yq11 or Yq12 region (Shapiro, 1991).

Genes affected by these translocations have not been identified, but probably include those in the AZF region (Vogt, 1998) of the Y chromosome (Delobel et al., 1998). This region has been divided into three subregions (AZFa–c; Table I), where genetic defects have been associated with different grades of impairment and arrest of spermatogenesis (Vogt et al., 1996; Vogt, 1998). Translocations between the Y chromosome and autosomes have an adverse effect on spermatogenesis due to interference with sex chromosome pairing and incomplete inactivation of the X chromosome (Handel and Hunt, 1992).

Translocations involving the Y chromosome cause severe disorders in spermatogenesis, but the cellular lesion in germ cell differentiation cannot be established from the site of a translocation (Delobel et al., 1998). Analysis of testicular biopsies suggests that translocations of the Y chromosome explain multiple disorders in spermatogenesis, ranging from failure of spermatogonial differentiation to quantitative losses in the formation of postmeiotic germ cells (Chandley et al., 1986; Chandley and Speed, 1995).

Other Y chromosome abnormalities disrupting spermatogenesis include the dicentric short arm (Chandley et al., 1986; Taniuchi et al., 1991) and ring formation involving the loss of large parts of the Yq arm, a region that encompasses most of the AZF region (Figure 2). Spermatogenesis typically fail to differentiate in patients with a dicentric short arm Y chromosome or Y ring formation, and certain patients may have short stature or tooth anomalies, suggesting that genes other than those directing the differentiation of germ cells are affected (Chandley et al., 1986; Chandley, 1994; Graves, 1995; Grootegoed et al., 1995).

No known therapeutic treatments are available for patients with Y chromosome translocations or malformations. Germ cell differentiation is arrested prior to the formation of spermatids in most cases, but elongated spermatids have been recovered from testicular biopsies in some patients and used for ICSI. Such practice must be viewed with caution given new evidence from some studies pointing to developmental delays in children following use of ICSI (Andrews et al., 1998; Bowen et al., 1998), although such delays were not apparent in other data sets using similar methods (Bonduelle et al., 1998c).

Deletions and mutations of the SRY gene. SRY is required for the regression of Müllerian ducts and the subsequent formation of testes, two sequential steps in male sexual differentiation. SRY is located on the short arm of the Y chromosome at Yp11.3 (Figure 2), adjacent to the pseudoautosomal boundary on interval I (Page et al., 1987; Sinclair et al., 1990). Over 26 inactivating point mutations in SRY have been discovered so far, and all are associated with gonadal dysgenesis or sex reversal syndromes or both (Pringle and Page, 1997). Point mutations in SRY produce structural and functional effects similar to those seen following deletion or translocation.

The SRY gene spans over 35 kilobase pairs, with an HMG-box region crucial for gene function. Mutations in the HMG-box region of SRY have the most deleterious impact on the male phenotype compared with mutations in other areas of the gene. SRY is absent in some patients due to deletion or translocation to the X chromosome during paternal meiosis. Paternal translocation of SRY to the X chromosome during meiosis results in either XY-female or XX-male genotypes in the offspring (Table I).

SRY encodes for a DNA-binding protein and is transcribed as a single exon (Cameron and Sinclair, 1997). This protein functions as a transcription factor regulating the formation of the testis in fetal development. Either the absence of SRY or a gene mutation explains sex reversal syndromes like XX-males and XY-females. Testes may fail to differentiate in pa-
patients with a 46, XY complement of chromosomes and normal SRY gene expression, indicating that genes from other chromosomes are required for spermatogenesis and the differentiation of the male phenotype (Page et al., 1987).

Deletion or translocation of SRY during paternal meiosis typically results in a feminine phenotype with gonadal dysgenesis in spite of a normal male 46, XY complement of chromosomes. Dysplasia of ovaries and an undifferentiated female genital tract are present, with underdeveloped but feminine secondary sexual features such as breasts, body proportions and external genitalia.

A similar consequence emerges in people who are phenotypically male but have a normal female complement of chromosomes, 46,XX, referred to as XX-male syndrome. Translocation of SRY to an X chromosome during paternal meiosis occurs in ~2% of patients with severe disorders in spermatogenesis (Nieschlag et al., 1997). Patients with an XX-male genotype have masculine secondary sexual features and gynaecomastia is common. Body height and weight is frequently below normal and typically feminine. The testes of XX-males are diminutive and the seminiferous epithelium is devoid of elongated spermatids. The prevalence of this condition among infertile patients underscores the importance of undertaking a complete genetic evaluation before considering the use of ICSI.

**Deletions in the Yq11 (AZF) region.** Major deletions in the euchromatic part of the Y chromosome interfere with spermatogenesis, as demonstrated by severe oligozoospermia and azoospermia (Tiepolo and Zuffardi, 1976; Hargreave et al., 1996). The euchromatic part of the Y chromosome contains a family of genes important for normal spermatogenesis, commonly referred to as AZF (azoospermia factor; Figure 2). Deletions occur in three different regions of Yq11 referred to as AZFa to AZFc and appear to be linked to specific lesions in spermatogenesis (Vogt, 1997, 1998). The incidence of deletions in the Yq11.21–23 region (Figure 2) among patients with severe disorders in spermatogenesis is estimated to range between 7 and 20% (Nakahori et al., 1996; Bonhoff et al., 1997; Roberts, 1998; Simoni et al., 1998). The relatively high occurrence of this disorder points to the advantage of adopting recombinant DNA techniques to assess genetic errors among infertile patients with apparent idiopathic infertility (Najmabadi et al., 1996; Qureshi et al., 1996).

Several genes have been proposed as AZF candidates based on microdeletion mapping in men with severe disorders in spermatogenesis (Vogt et al., 1992; Vogt, 1997). The number and location of AZF genes are a topic of intense investigation, as is their potential interaction within and among genes located on intervals 5 and 6 of the Y chromosome (Roberts, 1998; Simoni et al., 1998; Figure 2). Some 15 genes have been proposed as AZF candidates so far. Microdeletions appear to map to three different regions of Yq11.21–23 within intervals 5 and 6 (AZFa, AZFb, AZFc; Figure 2) (Vogt et al., 1995; Pryor et al., 1997; Roberts, 1998; Vogt, 1998). It remains to be elucidated if disorders in spermatogenesis can result from a single defective candidate gene or if the extinction of genetic information from a whole region is required (Vogt, 1998). Microdeletions in the Yq11.21–23 region occur as de-novo mutations since they are usually absent from the paternal genome (Vogt, 1998).

Severe disorders in spermatogenesis involve microdeletions at the start of Yq11.21 (AZFa) distal of DYS3 (Table I and Figure 2), but candidate genes have not been reported in this region (Vogt et al., 1995; Vogt, 1997; Roberts, 1998). De-novo deletion of genes in the Yq11.21 region (AZFa: Figure 2) causes the complete loss of germ cells (Table I), typical of the Sertoli cell-only syndrome (Vogt et al., 1995; Roberts, 1998; Vogt, 1998).

Within the Yq11.22–23 region (AZFb; Figure 2), four candidate genes (RBMI, SMCY, TSPY and EIF1AY) with suspected implications for spermatogenesis have been identified (Delbridge et al., 1997; Vogt, 1998). RBMI (RNA binding motif 1) and multiple repeats of the RBM gene family are distributed on the Y chromosome (Ma et al., 1993; Delbridge et al., 1997). Deletion of regions containing either RBMI, SMCY or the others leads to the early arrest of spermatogenesis at the level of pre- and post-meiotic spermatocytes, based on histological assessments of the testis (Delbridge et al., 1997). Ejaculates from patients with deletions in the AZFb region are most commonly azoospermic, but severe oligozoospermia may be encountered (Table I). No known mechanism has been proposed to explain why spermatogenesis can proceed in a few cases despite the absence of candidate genes (Nakahori et al., 1996; Delbridge et al., 1997).

The Yq11.22–23 region also contains AZFc (Figure 2), with important genes that appear to regulate spermatogenesis: DAZ (deleted in azoospermia) on interval 6 between DYS7C and DYS233 (Reijo et al., 1995, 1996), and SPGY (spermatogenesis gene on the Y, also referred to as DAZ2) located distal of DAZ. Recently, two new candidate genes have been discovered, TTY1 and TTY2, but their translation in germ cell development has not been confirmed. DAZ and SPGY (DAZ2; Figure 2) share remarkable structural homologies, as indicated by identical 72 basepair exons, but appear to function as independent genes of the same family (Habermann et al., 1998; Vogt, 1998). Patients with deletions in DAZ and SPGY (AZFc) present a range of spermatogenic disorders (Reijo et al., 1995, 1996; Nakahori et al., 1996; Pryor et al., 1997; Roberts, 1998). Analysis of the seminiferous epithelium, following testicular biopsy, reveals quantitative reductions in spermatogenesis or the arrest of germ cell development at the level of spermatids (Table I). Azoospermic or severe oligozoospermic ejaculates confirm lesions predicted by the morphological assessment of biopsied tissue (Shinka and Nakahori, 1996).

Proteins encoded by DAZ and SPGY (DAZ2) were identified exclusively in late spermatids, suggesting that these proteins have a role in the storage and transport of mRNA within
late spermatids (Habermann et al., 1998). Deletions of DAZ and DAZ2 interfere with the maturation of spermatids, but not with the development of spermatogonia or the formation of spermatocytes (Habermann et al., 1998). DAZL1 resides on chromosome 17 in mice and functions to restrict transcription in pre-meiotic spermatocytes, implying a regulatory role in early germ cell differentiation in the mouse (Niederberger et al., 1997). The human autosomal homologue of DAZL1 resides on chromosome 3p (DAZLA), but the function of this autosomal copy in spermatogenesis is unknown.

New microdeletions have been described on subinterval 6e of Yq (Figure 2), though no specific genes have been identified (Nicolai et al., 1998). Tentative indications, based on a cohort of patients with microdeletions in this region, suggest that spermatogenesis is impaired, as shown by oligozoospermia among all patients studied (Nicolai et al., 1998).

Genes in the Yq11.21–23 region (Figure 2) appear to share similar functions in directing spermatogenesis since they regulate the transcription of other genes involved in the development of germ cells (Jaffe and Oates, 1994). Some of these genes encode for RNA-binding proteins and are structurally similar to other human RNA-binding proteins (Delbridge et al., 1997), while others function as translation initiators and proteases. Certain genes, such as RBM1, are only transcribed in the testis at specific stages of germ cell development (Delbridge et al., 1997). The molecular site of action of such genes is unknown, since the cohort of genes regulated by those in the Yq11.21–23 region has not been established. Assessment of the role of genes that direct germ cell differentiation will be difficult, because the number of genes required for human spermatogenesis is large and the products of multiple genes are likely to interact to direct one or more steps in spermatogenesis (Engel et al., 1996).

Interactions among genes within Yq11.21–23 are not yet known. Understanding the functional significance of these interactions is important, but deletion of one gene critical to spermatogenesis could result in a range of disturbances unrelated to the lesions triggered by other candidate genes. Neither point mutations in any of the candidate genes within the Yq11.21–23 region are known at this time nor has one candidate gene been confirmed as AZF (Vogt, 1998). Detection of mutations in candidate genes could offer new insights into the impact of genes within the Yq11.21–23 region.

Deletions of genes in the Yq11.21–23 region of the Y chromosome are not associated with other known somatic disorders or defects in sexual differentiation despite their profound influence on spermatogenesis (Jaffe and Oates, 1994, 1997). Circulatory concentrations of FSH in patients with gene deletions in Yq11.21–23 are usually elevated or remain in the normal range (Kremer et al., 1997). Elevated FSH secretion coincides with the reduced inhibin production by Sertoli cells and aplastic seminiferous tubules (Roberts, 1998).

No therapeutic options are available to restore spermatogenesis among patients with microdeletions in Yq11.21–23. ICSI can be employed in the fraction of patients in whom the testis contains elongated spermatids, since a few spermatozoa may be recovered following testicular biopsy (Bonhoff et al., 1997). Male offspring are infertile due to the absence of genes on Yq11.21–23 which must be expressed to allow spermatogenesis to proceed. Physicians should make their patients aware of the risk of transmitting infertility to prospective male offspring or inducing other developmental disorders in both genders (Andrews et al., 1998; Bonduelle et al., 1998b) before assisted reproductive techniques are adopted.

**Autosomes**

Disorders in spermatogenesis cannot be predicted from the cytological analysis of chromosomal structure for two reasons. First, chromosomal aberrations differ from individual to individual since different sets of genes can be involved due to slightly different breakpoints and impact on different genes that cannot be assessed by cytological techniques. Second, neither deletions nor mutations in a gene can be established at the level of the light microscope. Cytogenetic analyses of chromosomes from fertile and infertile men indicate that autosomal translocations occur ~4–10 times more frequently in infertile than in fertile men (Chandley et al., 1975; Elliott and Cooke, 1997). The most typical structural aberration involves pericentric inversions on the following chromosomes: 1, 3, 5, 6, 9, 10 or 21 (Meschede et al., 1994). Even if all of the genes required for spermatogenesis could be detected by molecular techniques, the defects attributable to a single gene on an autosomal or sex chromosome would be difficult to resolve since hundreds of genes have a role in spermatogenesis (Engel et al., 1996). However, a coincidence of autosomal translocations and infertility must be considered since genes affected by these translocations are not known in most cases (Meschede et al., 1994).

The impact of autosomal translocations on spermatogenesis typically involves the formation of symaptosomal complexes in the early pachytene stage of spermatogenesis (de Perdigo et al., 1991; Guichaoua et al., 1992). Normal homologous synapsis in meiotic cells supports effective crossing over between homologous pairs, directing proper disjunction in the anaphase of meiosis (Guichaoua et al., 1992). Following autosomal translocations, asynaptic and heterosynaptic complexes are formed between non-homologous chromosomes and, most importantly, with the sex chromosome bivalent. Interactions between heterosynaptic quadrivalents and the XY pair purportedly interfere with the X chromosome inactivation required for proper meiosis (Johannisson et al., 1993). Transcription of X genes during the pachytene stage of meiosis can interrupt the meiotic cycle, as shown by sperma-
togenic arrest in patients with different types of translocated autosomes (de Perdigo et al., 1991; Johannisson et al., 1993).

**Robertsonian translocations**

Translocations between acrocentric chromosomes occur following centromere fusion and result in the loss of a centric fragment (Mak and Jarvi, 1996; Nieschlag et al., 1997). Robertsonian translocations are among the most common structural rearrangements in humans, where they involve chromosomes 13 and 14 in ~15 per 1000 births (Uccellatore et al., 1983). The cellular impact of Robertsonian translocations on spermatogenesis is unpredictable, but it appears to depend on the extent of trivalent/XY-pair formation during the pachytene stage (Johannisson et al., 1993). Pairing seems to interfere with the X chromosome inactivation required for normal spermatogenesis (Handel and Hunt, 1992). Alterations of the X chromosome may be a precondition for the formation of pairing sites between the Robertsonian trivalent and the X chromosome, thus explaining phenotypic differences in spermatogenesis and other traits among individuals with similar Robertsonian translocations.

Deleted genes attributable to the loss of centric fragments remain to be identified (Johannisson et al., 1993). Spermatogenic arrest, at the spermatocyte stage, is most common, but defects in spermatogenesis range from severe losses of spermatogonia to little or no change in the seminiferous epithelium (Johannisson et al., 1993; Meschede et al., 1998). Remarkably, the same Robertsonian translocations have been reported in fathers and sons with no apparent effect on spermatogenesis or fertility in the fathers while spermatogenesis was impaired in the sons (Johannisson et al., 1993). The high incidence of Robertsonian translocations in humans suggests that patients with such translocations are found frequently among infertile men (Meschede et al., 1998). FSH may be elevated in conjunction with this spermatogenic disorder, as is typical of patients with disorders in spermatogenesis (Uccellatore et al., 1983). Treatment strategies rely on ICSI provided that elongated spermatids or spermatozoa are present.

**Peri- and paracentric inversions**

Peri- and paracentric inversions are the least frequently occurring chromosomal rearrangements in humans, with an incidence of <0.01% in adult men. The occurrence among infertile men appears to be higher, but patients with inversions are rarely observed in infertility clinics.

Inversions of both types have been described in infertile patients on chromosomes 1 (Meschede et al., 1994), 7 (Navarro et al., 1993), 3, 6, 13, 20 and 21 respectively (Gabriel-Robez et al., 1988). Pericentric inversions involve the centromere, while paracentric inversions take place on the p or q arm of the affected chromosome. The current consensus is that pericentric inversions produce a greater incidence of unbalanced complements of chromosomes based on sperm karyotypes. This finding suggests that offspring of such patients have a higher probability of carrying an unbalanced complement of chromosomes, with consequent genetic defects occasioned by the loss or duplication of multiple genes (Navarro et al., 1993).

Inversions interfere with spermatogenesis, but the specific genes have not been identified (Mak and Jarvi, 1996). The nexus between inversions and spermatogenic disorders is based on a few cases, making it difficult to develop any generalization (Meschede et al., 1994). The impact of pericentric and paracentric inversions on spermatogenesis varies from patient to patient. Arrest at the spermatocyte stage has been described for a particular pericentric inversion on chromosome 1 (p34q23) (Meschede et al., 1994), whereas pericentric inversions involving other chromosomes have been associated with severe oligozoospermia or azoospermia (Meschede et al., 1998). Neither sexual differentiation nor other somatic tissues appear to be affected by inversions of either type.

Therapy depends on the extent of the pathophysiological lesion in spermatogenesis. The risk for inheriting unbalanced complements of chromosomes is higher in the offspring of patients with inversions, and the probability of spontaneous abortion should be anticipated when fertilization is achieved by ICSI or IVF (Navarro et al., 1993; Andrews et al., 1998).

**Campomelic dysplasia**

The gene for campomelic dysplasia has been identified as SOX-9 (SRY-type HMG-box gene 9) on chromosome 17. SOX-9 was the first sex-determining gene to be discovered on autosomes. Deletion, translocation and inactivating mutations involving SOX-9 cause campomelic dysplasia, including sex reversal in individuals with a normal male karyotype, 46, XY (Pringle and Page, 1997). The typical features of campomelic dysplasia and sex reversal have been observed in patients with translocations at breakpoints located in the q24–25 region of chromosome 17. Similar consequences result when breakpoints are localized outside the coding sequence of SOX-9. Translocations, mutations and deletions all appear to involve a defect in the promoter element for SOX-9, but a molecular explanation for the error in promoter activity is still uncertain. Disorders in SOX-9, originating at any level, cause a semithal, recessive disorder with a range of skeletal defects. Patients typically have feminine external genitalia with multiple somatic defects (Nieschlag et al., 1997). Testes are present as anlagen with few if any Sertoli or germ cells.

Patients with campomelic dysplasia are not seen in infertility clinics but residual testes, in the inguinal or abdominal
position, should be removed surgically to limit the risk of malignancies.

**Autosomal translocations**

Some 20 different balanced and unbalanced chromosomal translocations associated with disorders in spermatogenesis involve autosomes and more are expected to be discovered (Table II). Nearly all autosomes implicated in balanced or reciprocal translocations can be associated with infertility (Meschede et al., 1998).

**Table II.** Summary of balanced translocations reported to impair spermatogenesis and fertility. $t(\ldots)\ldots = t(\text{affected chromosomes})$ (cytogenetic location)

<table>
<thead>
<tr>
<th>Translocations between autosomes</th>
<th>Cytogenetic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t(1;9)\ (q32.1; q34.3)$</td>
<td></td>
</tr>
<tr>
<td>$t(1;16)\ (q22; p12)$</td>
<td></td>
</tr>
<tr>
<td>$t(1;16)\ (q23; p13)$</td>
<td></td>
</tr>
<tr>
<td>$t(1;18)\ (p31.2; q23)$</td>
<td></td>
</tr>
<tr>
<td>$t(1;19)\ (p13; q13), 1qh+$</td>
<td></td>
</tr>
<tr>
<td>$t(2;4)\ (q23; q27)$</td>
<td></td>
</tr>
<tr>
<td>$t(2;8)\ (q23; p21)$</td>
<td></td>
</tr>
<tr>
<td>$t(2;22)\ (q33; q22)$</td>
<td></td>
</tr>
<tr>
<td>$t(3;4)\ (p25; q23.3)$</td>
<td></td>
</tr>
<tr>
<td>$t(3;5)\ (q29; q14)\ (p12; q34)$</td>
<td></td>
</tr>
<tr>
<td>$t(3;14)\ (q27; q11)$</td>
<td></td>
</tr>
<tr>
<td>$t(3;20)\ (q11; p13)$</td>
<td></td>
</tr>
<tr>
<td>$t(4;6)\ (q26; q27)$</td>
<td></td>
</tr>
<tr>
<td>$t(4;13)\ (q11; q12.3)$</td>
<td></td>
</tr>
<tr>
<td>$t(5;8)\ (q22; q24.1)$</td>
<td></td>
</tr>
<tr>
<td>$t(9;12)\ (q22; q22)$</td>
<td></td>
</tr>
<tr>
<td>$t(9;13)\ (q22; q32)$</td>
<td></td>
</tr>
<tr>
<td>$t(9;15)\ (q22; q15)$</td>
<td></td>
</tr>
<tr>
<td>$t(9;17)\ (q11.3; q11.3)$</td>
<td></td>
</tr>
<tr>
<td>$t(9;17)\ (q11; q11)$</td>
<td></td>
</tr>
<tr>
<td>$t(9;20)\ (q34; q11)$</td>
<td></td>
</tr>
<tr>
<td>$t(10;14)$</td>
<td></td>
</tr>
<tr>
<td>$t(11;15)\ (q25; qter; pter)$</td>
<td></td>
</tr>
<tr>
<td>$t(13;14)$</td>
<td></td>
</tr>
<tr>
<td>$t(14;21)\ (q13; p13)$</td>
<td></td>
</tr>
<tr>
<td>$t(14;22)\ (p11; q11.1)$</td>
<td></td>
</tr>
<tr>
<td>$t(17;21)\ (p13; q11)$</td>
<td></td>
</tr>
<tr>
<td>$t(19;22)\ (p13.1; q11.1)$</td>
<td></td>
</tr>
</tbody>
</table>

**Translocations between autosomes and sex chromosomes**

<table>
<thead>
<tr>
<th>Genomic Location</th>
<th>Cytogenetic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t(13q;15q)/46, XY$</td>
<td></td>
</tr>
<tr>
<td>$t(Y;1)\ (q12; p34.3)$</td>
<td></td>
</tr>
<tr>
<td>$t(Y;3)\ (q12; p21)$</td>
<td></td>
</tr>
<tr>
<td>$t(Y;11)\ (q11.2; q24)$</td>
<td></td>
</tr>
</tbody>
</table>

**Translocations between sex chromosomes**

<table>
<thead>
<tr>
<th>Genomic Location</th>
<th>Cytogenetic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t(X;Y)$</td>
<td></td>
</tr>
<tr>
<td>$t(X;Y)\ (p22.3; q11)$</td>
<td></td>
</tr>
</tbody>
</table>

Distinct balanced translocations associated with infertility are limited to a few patients. The basis for impaired spermatogenesis in men with autosomal translocations has begun to emerge (Debiec-Rychter et al., 1992). Translocations appear to interfere with the inactivation of the X chromosome, as indicated by the pairing of autosomal quadrivalents with either the X or the Y chromosome during meiosis (Guichaoua et al., 1992). A significant correlation was observed between the extent of this interference and the grade of spermatogenic disorder (Micic and Micic, 1984; Gabriel-Robez et al., 1986, Luciani et al., 1987; Guichaoua et al., 1992). Other groups could not identify interference of the autosomal quadrivalents (de Perdigo et al., 1991) and hexavalents (Saadallah and Hulton, 1985) with the XY configuration. However, neither genes nor gene regulators that contribute to the disorder in spermatogenesis have been identified in infertile patients with balanced translocations, so the mechanism by which balanced translocations interfere with germ cell differentiation is not understood.

Therapeutic protocols rely on ICSI. Balanced and unbalanced chromosomal translocations, however, may be passed to offspring, posing an ethical dilemma for physicians treating these patients.

**Noonan’s syndrome**

Noonan’s syndrome is a congenital disorder of which half of all cases are due to autosomal dominant inheritance while the remainder occur spontaneously. The incidence is estimated at one per 1000 to one per 5000 live births. The hallmarks of this disorder include short stature, congestive heart failure, and a facial phenotype with posteriorly rotated ears (Allanson, 1987).

The gene defect has been localized to chromosome 12q22-qter between D12S84 and D12S366 for the autosomal form of the disorder (Jamieson et al., 1994). Other candidate loci have been proposed, but linkage analysis failed to prove the involvement of these loci (Jamieson et al., 1994).

The remarkably high incidence of Noonan’s syndrome should be considered in the evaluation of infertility patients. Interindividual variation in germ cell differentiation among Noonan’s syndrome patients is too broad to provide any systematic classification of the lesion in spermatogenesis. Primary hypogonadism occurs as a facultative feature. Hormonal therapy is indicated for cases including hypogonadism and late onset puberty. Patients should be informed about the genetic basis of their disease and the possibility that the trait can be transferred to offspring if spermatogenesis is stimulated or ICSI is used. Patients with Noonan’s syndrome should be examined for cryptorchidism, since ~60% of affected males are bilaterally cryptorchid (Allanson, 1987). Surgical relocation of the testes to the scrotum should be performed to reduce the risk of testicular malignancy.
Table III. Numerical aberrations in sex chromosomes and their impact on spermatogenesis

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Disorder</th>
<th>Phenotype</th>
<th>Gonadal manifestation</th>
<th>Extra-gonadal manifestation</th>
<th>Spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>47,XXY</td>
<td>Klinefelter’s</td>
<td>male</td>
<td>hypogonadism, defect in</td>
<td>gynaecomastia, tall stature</td>
<td>germ cells absent or</td>
</tr>
<tr>
<td></td>
<td>syndrome</td>
<td></td>
<td>spermatogenesis</td>
<td></td>
<td>early spermatogenic arrest,</td>
</tr>
<tr>
<td>48,XXXY</td>
<td>more X and mosaics</td>
<td></td>
<td></td>
<td></td>
<td>few if any spermatozoa</td>
</tr>
<tr>
<td>47,YYY</td>
<td>XYY-male</td>
<td>male</td>
<td>impaired spermatogenesis</td>
<td>none</td>
<td>all stages of spermatogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>possible</td>
<td></td>
<td>present but cell proliferation</td>
</tr>
<tr>
<td>46,XX+SRY</td>
<td>XX-male</td>
<td>male</td>
<td>hypospadias, cryptorchidism</td>
<td>gynaecomastia, feminine stature</td>
<td>variable</td>
</tr>
<tr>
<td>(SRY transl.)</td>
<td></td>
<td></td>
<td>intersexual genitalia, sex-</td>
<td>multiple extra-gonadal lesions</td>
<td></td>
</tr>
<tr>
<td>XY/X0</td>
<td>mixed gonadal</td>
<td>male only</td>
<td>reversal with abdominal testes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mosaic</td>
<td>dysgenesis</td>
<td>exceptional cases</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kartagener’s syndrome and axoneme defects

The genetic basis of Kartagener’s syndrome has not been elucidated. It appears to be inherited as an autosomal recessive mutation in most cases, but a dominant inheritance pattern has been established (Nieschlag et al., 1997). Patients with the syndrome lack dynein, a protein connecting triplets of microtubules within the axoneme. Dynein is required for the movement of cilia in all tissues where they are found, including spermatozoa. Spermatozoa produced by patients with this syndrome are immotile and the patients are infertile. Definitive diagnosis relies on demonstrating by electron microscopy that dynein connections between the microtubules are absent, since the spermatozoa appear normal in every respect (Nieschlag et al., 1997).

Infertility, bronchiectasis and situs inversus are the hallmarks of Kartagener’s syndrome. Cilia of the respiratory tract are affected and patients experience recurrent infections and formation of bronchiectasis.

A second axoneme defect affects the central doublet of microtubules. This defect, known as the 9+0 syndrome, is of unknown genetic origin. Affected patients produce spermatozoa that are immotile and incapable of fertilization (Nieschlag et al., 1997).

Assisted reproductive techniques rely on ICSI. Neither intrauterine insemination (IUI) nor IVF is effective, since spermatozoa from patients with Kartagener’s syndrome and other axoneme defects fail to fertilize in vitro. The risk of transmitting this defect via ICSI is 50% for the autosomal dominant forms.

Globozoospermia

Globozoospermia is attributed to a defect in the formation of the Golgi apparatus in spermatids and the eventual development of an acrosome. Spermatozoa from fertile men appear to have elongated heads when viewed by light and electron microscopy, while those from patients with globozoospermia are round and fail to bind to the zona pellucida (Schill, 1991). ICSI is the therapeutic option of choice for patients with globozoospermia, since spermatozoa are easily prepared from the ejaculate.

Numerical aberrations in sex chromosomes

Accessory chromosomes arise because of errors in parental meiosis. Parental non-disjunction of sex chromosomes can result in numerical aberrations of chromosomes, 47+, with multiple X or Y chromosomes (Table III). The functional consequences of accessory sex chromosomes are mild compared with the effects occasioned by numerical aberrations in an autosome. Loss of a sex chromosome such as the X chromosome, as indicated by a 45– genotype, produces multiple somatic defects and profound disturbances in sexual differentiation of females (Turner syndrome, 45, X0).

Klinefelter’s syndrome: 47,XXY

Klinefelter’s syndrome is the most common chromosomal defect causing hypogonadism and infertility in men (Nieschlag et al., 1997). The hallmarks of Klinefelter’s syndrome include hypogonadism with mild to severe losses in spermatogenesis, tall stature, gynaecomastia and obesity with a feminine pattern of fat distribution (Klinefelter et al., 1942; Klinefelter, 1984). Klinefelter’s patients have one or more accessory X chromosomes (Table III). The additional X chromosome arises from the non-disjunction of parental germ cells during meiosis (Klinefelter et al., 1942; Klinefelter, 1984). About two-thirds of meiotic non-disjunctions are of
maternal origin and about one-third are paternal (Nieschlag et al., 1997). The typical karyotype is 47, XXY, but chromosomal mosaics with 46, XY/47 XXY and complements with multiple X chromosomes like 48, XXXY are known (Zang, 1984). The incidence of Klinefelter’s syndrome is one per 500–700 male births.

The molecular basis for hypogonadism in Klinefelter’s patients is unclear. Klinefelter’s patients with a mosaic karyotype of 46, XY/47 XXY have been known to induce spontaneous pregnancies, and even patients with the typical form of Klinefelter’s syndrome have fathered children in exceptional cases (Schill et al., 1984). The seminiferous epithelium of patients with a mosaic karyotype including a 46,XY cell line has mild defects in spermatogenesis (Gordon et al., 1972; Schill et al., 1984). Testes of patients with both a 46,XY or 47, XXY chromosomal complement undergo meiosis to form spermatozoa with either a normal, 23, X or Y, or abnormal, 24,XY, karyotype (Cozzi et al., 1994; Foresta et al., 1998). Sperm karyotypes of Klinefelter’s patients indicate that 2–20% of spermatozoa have sex chromosome aneuploidy (Foresta et al., 1998).

In patients with the typical form of Klinefelter’s syndrome, the testicular volume is 80–90% below normal and ejaculates contain few or no spermatozoa (Schill et al., 1984). The seminiferous tubules are fibrotic and hyalinized, with infrequent spermatogonia (Bandmann and Perwein, 1984). The spermatogonia fail to differentiate beyond the primary spermatocyte stage of spermatogenesis, but spermatogenesis can proceed to completion, at least in a few areas of the germinal epithelium.

The diagnosis of Klinefelter’s syndrome is often delayed until adult patients consult an infertility specialist. The therapeutic strategies available to patients with Klinefelter’s syndrome include those adopted for hypogonadal men (Nieschlag et al., 1997). Secondary sexual characteristics are unaffected and spermatogenesis is normal among most XYY-men since the extra Y chromosome is eliminated during meiosis. Incomplete inactivation of the X chromosome during the pachytye stage of meiosis is the genetic explanation for the arrest in spermatogenesis that is occasionally observed in affected patients (Handel and Hunt, 1992).

Patients with an XYY genotype, however, produce spermatozoa with disomic and hyperhaploid karyotypes, such as 24,XY or 24,YY at a much greater rate than is observed in individuals with a normal complement of 46 chromosomes (Blanco et al., 1997; Chevret et al., 1997). The risk of sex chromosome trisomies in the offspring of such patients is enhanced, but can be estimated on the basis of the number of hyperhaploid karyotypes in ejaculated spermatozoa (Blanco et al., 1997; Chevret et al., 1997). Infertile patients with this spermatogenic disorder can be assisted with ICSI, but consideration should be given to the genetic risks and ethical issues that stem from assisted reproductive protocols.

**XX-male syndrome: 46,XX+ SRY**

Approximately 2% of the men seen at infertility clinics have a 46,XX genotype (Nieschlag et al., 1997). Despite a normal female karyotype, these individuals have fully developed masculine features due to the presence of SRY in their complement of chromosomes (Tables I and III). SRY, a gene required for testicular development, and other genes in the pseudoautosomal boundary region of the Y chromosome (Figure 2) are partially translocated to an X chromosome during paternal meiosis, thus explaining this form of sex reversal (Pringle and Page, 1997).

SRY encodes for a nucleotide-binding protein directing the transcription of other genes required for the formation of a testis. Testicular volume is reduced, the seminiferous epithelium usually is devoid of germ cells and ejaculates are azoospermic, since the subset of genes on the Y chromosome that are required for spermatogenesis are absent (Cooper and Sandlow, 1996). The secondary sexual features of XX-males are fully differentiated, but gynaeomastia is common, with a feminine stature. Infertility in these patients is not treatable since spermatogenesis is arrested prior to the formation of spermatids.

**Mixed gonadal dysgenesis: mosaic 45, X0/46, XY**

Mixed gonadal dysgenesis occurs among patients with a mosaic arrangement of chromosomes such as 45, X0/46, XY (Table III). Gonadal and phenotypic sexual features are typically female, but testicles are retained in the abdomen and may occur unilaterally. In exceptional cases, males with abdominal
testes have been described, but both spermatogenesis and Leydig-cell function are reminiscent of that seen in the prepubertal state. Testosterone replacement therapy is required for the completion of sexual differentiation and the expression of a male phenotype. Remnants of testicular tissue should be removed surgically in patients with feminine secondary sexual features.

**Endocrine disorders of genetic origin and their impact on spermatogenesis**

The differentiation of the male phenotype, including the seminiferous epithelium, requires the secretion of FSH and LH from the pituitary gland and the production of testosterone by Leydig cells. Disorders of male sexual differentiation, including defects such as micropenis and hypospadias, occur when androgen is insufficient or absent during organogenesis (Table IV). Patients with a 46,XY karyotype may be genetic but not phenotypic males as judged by the failure of secondary sexual tissues to differentiate according to a male pattern. Spermatogenesis is not initiated when GnRH is lacking or when either FSH or LH secretion fails, resulting in testosterone insufficiency (Behre et al., 1997). The range of developmental disorders in spermatogenesis arising from a defect in one or more genes controlling endocrine function is summarized in Table IV and presented in more detail below.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Disorder</th>
<th>Gonadal manifestation</th>
<th>Extra-gonadal manifestation</th>
<th>Spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xp22.3</td>
<td>KAL-1</td>
<td>Kallmann's syndrome</td>
<td>hypotrophic testes, anosmia, cryptorchidism</td>
<td>renal dysplasia</td>
<td>spermatogenic arrest</td>
</tr>
<tr>
<td>15q11–13</td>
<td>IC</td>
<td>Prader–Labhardt–Willi syndrome</td>
<td>hypogonadism, hypogonitalism, hypospadias, skeletal anomalies</td>
<td>spermatogenic arrest at spermatid stage, normal fertility possible</td>
<td></td>
</tr>
<tr>
<td>Xp21.2–21.3</td>
<td>DAX-1</td>
<td>congenital hypoplasia of adrenals and testes</td>
<td>hypogonadism, adrenal insufficiency</td>
<td>spermatogenic arrest</td>
<td></td>
</tr>
<tr>
<td>11q13</td>
<td>BBS1</td>
<td>Laurence-Moon syndrome and Bardet-Biedel syndrome</td>
<td>hypogonadism, mental retardation, obesity, hypogonadism</td>
<td>wide range of defects in spermatogenesis, normal spermatogenesis possible</td>
<td></td>
</tr>
<tr>
<td>16q21</td>
<td>BBS2</td>
<td>congenital hypoplasia of adrenals and testes</td>
<td>hypogonadism, hypogonitalism, obesity, hypogonadism</td>
<td>quantitative reductions in germ cell proliferation</td>
<td></td>
</tr>
<tr>
<td>15q22.3</td>
<td>BBS3</td>
<td>congenital hypoplasia of adrenals and testes</td>
<td>hypogonadism, mental retardation, obesity, hypogonadism</td>
<td>quantitative reductions in spermatogenesis</td>
<td></td>
</tr>
<tr>
<td>3p12</td>
<td>BBS4</td>
<td>congenital hypoplasia of adrenals and testes</td>
<td>hypogonadism, mental retardation, obesity, hypogonadism</td>
<td>quantitative reductions in spermatogenesis</td>
<td></td>
</tr>
<tr>
<td>3p</td>
<td>PIT-1</td>
<td>congenital hypopituitarism of adrenals and testes</td>
<td>hypogonadism, mental retardation, obesity, hypogonadism</td>
<td>quantitative reductions in spermatogenesis</td>
<td></td>
</tr>
<tr>
<td>2p21-p16</td>
<td>FSHR</td>
<td>FSH receptor mutation</td>
<td>defective spermatogenesis, mental retardation, obesity, hypogonadism</td>
<td>quantitative reductions in spermatogenesis</td>
<td></td>
</tr>
<tr>
<td>10q24.3</td>
<td>CYP 17</td>
<td>17,20-desmolase/gonadal dysgenesis</td>
<td>extra-gonadal effects of hypogonadism</td>
<td>range of defects in spermatogenesis</td>
<td></td>
</tr>
<tr>
<td>9q22</td>
<td>HSD17B3</td>
<td>17α-hydroxylase defect</td>
<td>extra-gonadal effects of hypogonadism</td>
<td>range of defects in spermatogenesis</td>
<td></td>
</tr>
<tr>
<td>2p23</td>
<td>SRD5A2</td>
<td>5α-reductase 2 deficiency</td>
<td>gonadal dysgenesis, femininity</td>
<td>germ cells absent, Sertoli only</td>
<td></td>
</tr>
<tr>
<td>Xq11–12</td>
<td>AR</td>
<td>androgen receptor</td>
<td>femininity, abdominal testes</td>
<td>germ cells, Sertoli only</td>
<td></td>
</tr>
<tr>
<td>Xq11–12</td>
<td>AR</td>
<td>Reifenstein syndrome</td>
<td>cryptorchidism, femininity</td>
<td>spermatogenic arrest at spermatid level</td>
<td></td>
</tr>
<tr>
<td>Xq11–12</td>
<td>AR</td>
<td>Undervirilized fertile male syndrome</td>
<td>spermatogenic lesion, undervirilization</td>
<td>quantitative reductions in spermatogenesis possible</td>
<td></td>
</tr>
</tbody>
</table>
**Kallmann’s syndrome**

Kallmann’s syndrome is caused by a deletion or mutation in \( KAL-1 \) located on the X chromosome at Xp22.3 (Figure 2 and Table IV). Deletions or mutations in \( KAL-1 \) result in hypogonadotropic hypogonadism and anosmia and occur in one per 7000–8000 births (Behre et al., 1997). \( KAL-1 \) is susceptible to deletion along with other genes in Xp22-pter, as noted above in the section about the Xp22 contiguous gene syndrome. Deletions or mutations in \( KAL-1 \) have been described in patients with sporadic Kallmann’s syndrome, but families with X-linked recessive inheritance have been confirmed (Behre et al., 1997).

\( KAL-1 \) encodes for a cell adhesion protein and shares structural homology with other cell adhesion molecules made up of repeated sequences of fibronectin type III (Pringle and Page, 1997). The gene product interferes with the migration of GnRH neurons to the hypothalamus during brain development. Migration failure coincides with the lack of GnRH, the neural hormone responsible for the synthesis and release of LH and FSH from the anterior pituitary gland (Pringle and Page, 1997).

Typical patients with Kallmann’s syndrome have an almost total lack of FSH and LH, depending on the extent of GnRH insufficiency (Behre et al., 1997). The syndrome frequently coincides with anosmia because the same adhesion molecules guide the development of both hypothalamic GnRH and olfactory neurons (Bick et al., 1989). Other defects, such as unilateral renal aplasia or renal dysplasia, are facultative features of Kallmann’s syndrome (Table IV; Bick et al., 1989).

The male phenotype is normal in patients with Kallmann’s syndrome since testosterone concentrations are adequate to support sexual differentiation during organogenesis. All secondary sexual features are infantile due to the lack of the pituitary gonadotrophic hormones required for steroidogenesis. Affected patients retain the classical hallmarks of hypogonadism evident at puberty or in adult life. Diagnostic approaches rely on physical examination and are confirmed by near undetectable concentrations of FSH, LH and testosterone in peripheral blood (Behre et al., 1997). Definitive diagnosis is based on provocative infusion of GnRH and the detection of a brisk and robust increase in the secretion of both gonadotrophins and testosterone.

Spermatogenesis is absent or highly reduced depending on the severity of the hypothalamic deficiency in GnRH secretion. Phenotypic variations among adult patients with Kallmann’s syndrome range from normogonadotropic fertile patients to those reminiscent of prepubertal boys. A clear relationship between genotype and phenotype has not been established because mutations in \( KAL-1 \) involve over 10 different basepair deletions and mutations. Multiple genetic defects result in remarkably diverse phenotypes that defy systematic classification of the disorder at the level of spermatogenesis or other endocrinological endpoints (Quinton et al., 1996).

Kallmann’s syndrome accounts for ~5% of infertile men with hypogonadotrophic hypogonadism (Behre et al., 1997). Patients respond to induction of puberty with GnRH and testosterone therapy supporting the development of secondary male sexual features including erectile function and ejaculation. Spermatogenesis can be stimulated to near normal levels once patients wish to father children, as documented in multiple clinical experiences (Behre et al., 1997).

**Prader–Labhardt–Willi syndrome**

Prader–Labhardt–Willi syndrome is caused by the absence of a small part of a paternal chromosome. The syndrome occurs in one per 15 000 births and is associated with hypogonadotrophic hypogonadism including spermatogenic arrest and testicular atrophy (Table IV). The genetic defect may be caused by a deletion, mutation, uniparental disomy or translocation of parts of chromosome 15q11–13 (Robinson et al., 1998). Deletions and mutations involving chromosome 15q11–13 can impair the transcription of genes in the 1 megabase vicinity of the imprinting centre (Saitoh et al., 1996). Microdeletions causing gene defects in the imprinting centre on chromosome 15q11–13 appear to be the most frequent basis for this syndrome (Robinson et al., 1991)

Failure of the imprinting centre (Table IV) interferes with the expression of genes in the vicinity of paternal alleles (Lyko et al., 1998). Deletion mapping suggests that the deleted segment contains ~200 kilobases located between \( D I S S 1 3 \) and the first exon of \( S N R P N \). Multiple genes in the 1 megabase vicinity are subsequently repressed including \( Z N F 1 2 7 , S N R P N , I P W , P A R - 1 \) and \( P A R - 5 \) (Saitoh et al., 1996). The primary lesion caused by gene failure is located in the hypothalamus, where it appears to involve a defect in GnRH-releasing neurons. Pituitary gland involvement, as viewed by magnetic resonance imaging, has been established (Miller et al., 1996). Estimates of circulating concentrations of FSH, LH and testosterone confirm deficits in hypothalamic GnRH release.

Short stature, mental retardation, obesity and cryptorchidism are the dominant clinical features of Prader–Labhardt–Willi syndrome (Robinson et al., 1991, 1998). Testicular biopsies reveal that Leydig cells are undifferentiated and spermatogenesis is arrested at the level of late spermatids (Hamilton et al., 1972). The seminiferous epithelium is aplastic and disorganized due to the combined influence of hypogonadism and cryptorchidism (Wu et al., 1981). Hypogenitalism is common, but not a consistent feature, including micropenis, scrotal dysplasia and Leydig cell failure (Butler, 1990).

Testes should be translocated to the scrotum within the first 2 years of life if they remain in an inguinal or abdominal position. Treatment with pulsatile GnRH was shown to trigger...
FSH and LH release, with the subsequent induction of spermatogenesis, but fertility was not assessed in the treated subjects (Müller, 1997; Swaab, 1997). Rescue of the seminiferous epithelium is not a routine practice among patients with Prader–Labhardt–Willi syndrome since the disorder involves multiple somatic defects, including mental retardation.

**Congenital hypoplasia of adrenal and testes**

Congenital hypoplasia of the adrenal gland and testis is a rare condition affecting the development of steroidogenic tissues during organogenesis (Habiby et al., 1996). Multiple forms of primary adrenal insufficiency are known, with either X-linked or autosomal recessive inheritance and both can occur in association with hypogonadotropic hypogonadism (Partsch and Sippell, 1989; Nieschlag et al., 1997).

The X-linked form has been localized to the Xp21.2–21.3 region of the X chromosome, where two-thirds of affected patients express a mutation in DAX-1 (Table IV). Most defects in DAX-1 involve the cytomegacell form of the disorder typified by congenital adrenal hypoplasia and hypogonadotropic hypogonadism. Three types of defects have been identified in DAX-1: major deletions, single base substitutions and basepair deletions. The syndrome can also be inherited via maternal alleles, as indicated by affected families (Zanaria et al., 1994; Yanase et al., 1996).

*DAX-1* consists of two exons encoding for a member of the nuclear hormone receptor superfamily with a novel DNA-binding domain (Zanaria et al., 1994; Yanase et al., 1996). The gene product is a dominant negative regulator protein that functions in transcription and is mediated by the retinoic acid receptor (Zanaria et al., 1994). Inactivating mutations in *DAX-1* or the loss of *DAX-1* typically result in hypoplasia of the adrenals and testis (Lalli et al., 1997). Defects attributed to *DAX-1* are traceable to a failure in steroidogenic cell differentiation at the time that the anlagen for the adrenal glands and gonads develop from the genital ridge.

*DAX-1* expression also has implications for the differentiation of GnRH neurons in the hypothalamus and gonadotrophins of the anterior pituitary gland, but the mechanisms of *DAX-1* action in the hypothalamus and anterior pituitary are not yet known (Partsch and Sippell, 1989). Experimental investigations in the mouse indicate that *DAX-1* expression is required for the function of hypothalamic neurons that regulate the secretion of adrenocorticotropic hormone (ACTH) and gonadotrophic hormones in the pituitary gland (Habiby et al., 1996). It has not been elucidated whether *DAX-1* mutations primarily affect the hypothalamus or the pituitary gland or both (Habiby et al., 1996).

Congenital defects in the adrenal gland and testes, due to *DAX-1*, result in simultaneous deficiencies in glucocorticoids, mineralocorticoids and testosterone (Kletter et al., 1991). The adrenals of patients with a failure in *DAX-1* are severely hypoplastic and disorganized and usually go undetected in computed tomographic examinations. Most newborns die within the first 3 weeks unless supplemented with adrenal cortical hormones (Behre et al., 1997). Lifelong substitution of glucocorticoids and mineralocorticoids is required to replace hormones made by the adrenal cortex. Surviving, untreated males show classical features of Addison’s disease and hypogonadotropic hypogonadism requiring substitution of testosterone (Table IV). Secondary sexual features of males develop normally in most cases but cryptorchidism occurs frequently, owing to the relative lack of testosterone during fetal development (Kletter et al., 1991).

Spermatogenesis among patients lacking *DAX-1* is reduced or arrested completely (Table IV). The cellular architecture of the testis is disorganized due in part to the absence of testosterone (Kletter et al., 1991). Fertility is not a therapeutic objective among patients with congenital adrenal hypoplasia.

Autosomal recessive inheritance explains a variant of congenital adrenal hypoplasia not linked to *DAX-1* mutations (Muscatelli et al., 1994). Patients with the alternative form have mild to modest deficiencies in both adrenal and testicular steroidogenesis, but hypothalamic control of anterior pituitary hormone secretion is usually normal. Modest reductions in spermatogenesis are evident but patients usually are fertile. *FTZ1*, a gene encoding for the nuclear orphan receptor and transcription factor SF1 residing on chromosome 9q33, is a candidate gene for the alternative form of congenital adrenal hypoplasia since the mouse homologue (*Ftz-F1*) also interferes with the differentiation of steroidogenic tissues (Lala et al., 1992). Expression of *Ftz-F1* in mice indicates that it precedes the transcription of *DAX-1* and participates in sexual differentiation. The gene product SF1 itself seems to interfere with *DAX-1* transcription via direct binding to repressor regions of *DAX-1* (Nachtigal et al., 1998).

**Laurence–Moon and Bardet–Biedel syndromes**

Both syndromes are rare autosomal recessive disorders that involve a wide range of defects, including mental retardation, polydactyly, obesity, retinal pigmented dystrophy and hypogonitalism (Beales et al., 1997; Bruford et al., 1997). Hypogonadism is a facultative feature in both syndromes and spermatogenesis can be impaired in affected males (Whitaker et al., 1987). The clinical distinction between the less observed Laurence–Moon syndrome and the more prevalent Bardet–Biedel syndrome is based on polydactyly, a feature limited to the Laurence–Moon syndrome (Leppert et al., 1994).

The chromosomal origin and genetic basis for Laurence–Moon syndrome remain to be established. In sharp contrast, genetic defects in the Bardet–Biedel syndrome have been localized to four loci, BBS1 to BBS4, in families with hereditary Bardet–Biedel syndrome (Table IV). Most families show linkage to the BBS1 locus on chromosome 11q13 (Leppert et al., 1994; Bruford et al., 1997). Most families show linkage to the BBS1 locus on chromosome 11q13 (Leppert et al., 1994; Bruford et al., 1997).
et al., 1994), followed by defects in BBS2 on chromosome 16q21, BBS3 on chromosome 15q22.3–23 and BBS4 on chromosome 3p12 (Beales et al., 1997; Bruford et al., 1997). Approximately 50% of families do not present linkage to any BBS locus, implicating one or more additional loci in the disorder. Candidate genes within the BBS loci have not been isolated (Beales et al., 1997). BBS1 on chromosome 11q13 contains several genes of physiological importance such as PYGM (muscle-type glycogen phosphorylase), GCK (human B-lymphocyte serine/threonine protein kinase), and ZFMI (zinc finger protein) (Kedra et al., 1997). Apart from hypogonitalism, which is characteristic of both Laurence–Moon and Bardet–Biedel syndromes, other clinical features include mental retardation, obesity, neurological defects, renal abnormalities and retinal dystrophy (Whitaker et al., 1987). Hypogonitalism in patients with Laurence–Moon or Bardet–Biedel syndrome is typified by micropenis, hypospadias and cryptorchidism in males, but genital abnormalities, such as vaginal atresia, are also known in females (Stoler et al., 1995). The failure in pituitary gonadotrophin secretion impairs both steroidogenesis and spermatogenesis (Table IV). Infertility is common but not always present, since patients with normal testicular function and spermatogenesis are known to carry Laurence–Moon or Bardet–Biedel syndrome. Testicular biopsies contain elongated spermatids, but the number of spermatids that complete spermatogenesis is well below normal (Channmugam et al., 1977; Whitaker et al., 1987). The clinical management of Laurence–Moon and Bardet–Biedel syndrome is focused primarily on treating the multiple somatic defects and not on restoring spermatogenesis. In those instances when the somatic defects are mild, treatment for hypogonadism can be accomplished by hormone replacement. Spermatogenesis responds to gonadotrophin stimulation in some cases (Channmugam et al., 1977; Whitaker et al., 1987).

**Congenital hypopituitarism**

PIT-1, a transcription factor, is located on chromosome 3p (Raskin et al., 1996). Mutations in the PIT-1 gene contribute to the pathogenesis of congenital hypopituitarism (Table IV), but extra-pituitary effects of mutations in PIT-1 are known, indicating that PIT-1 regulates multiple genes (Pellegrini-Bouiller et al., 1996). Mutations affecting the DNA-binding domain of PIT-1 appear to have a profound impact on fetal neurons during pituitary differentiation (Pfäffle et al., 1996). PIT-1 is encoded by six exons on chromosome 3p (Raskin et al., 1996). PIT-1 regulates the expression of prolactin, growth hormone and the β unit of thyrotrophin by binding to the promoter region of genes for each of these peptide hormones (Pellegrini-Bouiller et al., 1996). Mutations in PIT-1 typically involve exons 5 and 6, causing structural and functional abnormalities in the DNA-binding domain.

Mutations in PIT-1 cause congenital hypopituitarism, with profound structural and functional alterations in the pituitary gland. The clinical phenotype of disorders in PIT-1 differs among patients, suggesting a multifactorial origin of the mutations. Aplasia is possible in certain patients, while in others fusion defects occur between the anterior and posterior lobes (Pfäffle et al., 1996). The clinical picture includes the almost total lack of growth hormone (GH), thyrotrophin (TSH) and prolactin, and thus the arrest of body growth early in life. Gonadotrophins are not affected in most instances, but patients who lack gonadotrophins are known (Nogueira et al., 1997). Insufficient stimulation by FSH and LH is believed to impair spermatogenesis (Nogueira et al., 1997). Congenital hypopituitarism is a rare disorder. No consensus has emerged for treating infertility in these patients since clinical experience is limited. Substitution of growth hormone and other critical hormonal peptides is required (Behre et al., 1997).

**Isolated deficiency of FSH and LH**

Isolated deficiency of FSH and LH occurs infrequently among patients seen at infertility clinics. Neither the chromosomal origin nor the genetic basis for isolated deficiencies in LH or FSH secretion is known (Table IV). GnRH-secreting neurons in the hypothalamus are implicated because replacement therapy with repeated but periodic GnRH evokes the production of both FSH and LH from the anterior pituitary gland (Al Ansari et al., 1984). Isolated FSH deficiency is a rare condition in which LH and testosterone concentrations remain in the normal range in infertile patients presenting with severe disorders of spermatogenesis. FSH release can be induced by repeated pulsatile treatment with GnRH. Differentiation of the male sexual phenotype is complete. Defects in spermatogenesis range from modest reductions in the number of ejaculated spermatozoa (Haegg et al., 1978) to a failure in spermatogenesis at the level of spermatocytes or spermatids (Maroulis et al., 1977). In certain patients, only Sertoli cells are present within the testis (Al Ansari et al., 1984). The wide range of differences in spermatogenesis among patients with isolated FSH deficiency points to the interaction of multiple gene products in this genetic disorder. Treatment can be accomplished by periodic but repeated administration of GnRH to evoke the secretion of FSH (Al Ansari et al., 1984) and restore spermatogenesis (Maroulis et al., 1977). Clinical success is limited to the fraction of patients in whom spermatogenesis has halted at the spermatid stage or where all classes of germ cells are present but markedly reduced. Isolated deficiency in LH secretion was found first among patients who also had Kallmann’s syndrome (Wortsman and Hughes, 1996). The hallmarks of isolated LH deficiency, also known as the Pasqualini or fertile eunuch syndrome, include hypogonadism with hypoplastic Leydig cells, subnormal con-
centrations of LH and testosterone, and normal concentrations of FSH (Table IV). All classes of germ cells are present, including spermatozoa in the ejaculate (Faiman et al., 1968; Williams et al., 1975). Spermatogenic failure is attributable to testosterone insufficiency. Despite the apparent hypogonadism, patients have been reported to father children (Faiman et al., 1968; Williams et al., 1975). Testosterone replacement in patients with isolated LH deficiency alleviates the symptoms of hypogonadism and may stimulate spermatogenesis (Behre et al., 1997). Therapeutic strategies to restore spermatogenesis have been attempted in exceptional cases of both disorders, but routine experience is missing (Wortsmann and Hughes, 1996).

Mutations of the FSH receptor

Mutations of the FSH receptor typically involve profound reductions in spermatogenesis (Tapanainen et al., 1997). The gene encoding for the FSH receptor, FSHR, resides on chromosome 2p21-p16 and consists of 10 transcribed exons (Table IV). An inactivating point mutation in FSHR causes a recessively inherited form of hypogonadotrophic failure in homozygous women, resulting in ovarian failure (Tapanainen et al., 1997). The same point mutation in exon 7 (566c to T) in homozygous men results in severely oligozoospermic ejaculates and elevated FSH concentrations. Screening tests for mutations of the FSH receptor in all exons in a group of infertility patients indicated that receptor failure is an uncommon cause of male infertility (Tuerlings et al., 1998b).

Genetic disorders of testosterone synthesis

17,20-Desmolase/17α-hydroxylase and 17β-hydroxysteroid dehydrogenase defect

Testosterone biosynthesis depends on the cytochrome P-450c17 enzyme, involving 17,20-desmolase and 17α-hydroxylase activity, and the P-450 enzyme 17β-hydroxysteroid dehydrogenase (Payne and Youngblood, 1995; Suzuki et al., 1998). Testosterone insufficiency, inducing disturbances in male sexual differentiation and infertility, has been attributed to a range of enzymatic defects involving the complete to partial lack of testosterone due to autosomal recessive mutations of steroidogenic enzymes (Table IV). More than 21 mutations are known in the gene for P-450c17 on chromosome 10q24.3, resulting in partial or no activity of 17,20-desmolase and 17β-hydroxylase (Monno et al., 1997; Suzuki et al., 1998). Over 14 mutations have been identified in the gene encoding for 17β-hydroxysteroid dehydrogenase III, the testis-specific isoenzyme, on chromosome 17 in patients with 17β-hydroxysteroid dehydrogenase deficiency (Andersson, 1995).

The differentiation of the male phenotype requires testosterone. Variable forms of male pseudohermaphroditism are evident among patients who fail to produce testosterone due to defects in 17,20-desmolase/17α-hydroxylase or 17β-hydroxysteroid dehydrogenase (Nieschlag et al., 1997). The extent of the enzyme defect and the level of testosterone insufficiency is variable, providing wide deviations in secondary sexual differentiation (Nieschlag et al., 1997). Testes remain in the abdomen or in the inguinal canal in patients with partial or complete enzyme defects. Germ cell development fails to proceed beyond the spermatocyte stage due to the absence of testosterone or elevated temperatures resulting from cryptorchidism. Testicles should be removed in patients with feminine sexual features or, in male patients, relocated surgically to the scrotum to reduce the risk of testicular cancer. Most patients with male pseudohermaphroditism have feminine genitalia (Table IV).

Androstenedione concentrations are elevated in post-pubertal patients with male pseudohermaphroditism due to a 17β-hydroxysteroid dehydrogenase deficiency (Anderson, 1995). Patients with the most severe forms of pseudohermaphroditism, occasioned by the complete to partial lack of testosterone during embryonic development, are usually not seen in infertility clinics.

Patients with abnormal male sexual differentiation and minor lesions such as mild hypospadias are rare (Yanase, 1995). Testosterone substitution is recommended from the time that puberty is anticipated in patients who have near normal secondary sexual features (Nieschlag et al., 1997). Spermatogenesis may be induced by testosterone replacement in the subset of patients who manifest modest losses in steroidogenic enzyme activity resulting in a normal male phenotype, but there is a lack of systematic therapeutic approaches.

5α-Reductase 2 deficiency

The conversion of testosterone to dihydrotestosterone is catalyzed by the enzyme 5α-reductase 2 (Table IV). Enzyme production is a prerequisite for the differentiation of the urogenital tubercle and sinus, the anlagen of male external genitalia. The gene encoding for 5α-reductase 2 is located on chromosome 2 and consists of five transcribed exons (Wilson et al., 1993). Either deletion or mutations of the gene SRD5A2 results in different grades of male pseudohermaphroditism (Meschede et al., 1997). Mutations in SRD5A2 affect all exons of the gene, resulting in a deficiency of 5α-reductase 2. The catalytic activity of the enzyme is reduced substantially following mutations (Wilson et al., 1993). A total of 28 inactivating mutations have been demonstrated in 52 families with this disorder (Wilson et al., 1993). All transcribed exons are affected and most of them involve point mutations with recessive inheritance (Wilson et al., 1993).

The external genitalia of newborns with 5α-reductase deficiency are feminized and most children are raised as females. The structural features of the disorder include perineoscrotal hypospadias with a pseudovagina (Meschede et al., 1997). The prostate gland is dysplastic, rudimentary, and located in a
post-urethral position (Meschede et al., 1997). Testes remain in the inguinal position, but seminal ducts and epididymides are evident since neither of these structures requires dihydrotestosterone for differentiation. Spermatogenesis is halted at early steps since germ cell differentiation fails at body temperature (Wilson et al., 1993).

Substitution therapy with either testosterone or dihydrotestosterone has been attempted, but current clinical experience is too limited to recommend a standard treatment. External genitalia may be enlarged and corrected surgically. No therapeutic approaches for infertility have been reported since the deficiency in 5α-reductase 2 is a rare form of male pseudohermaphroditism limited to about 50 families (Wilson et al., 1993). Testes should be removed from patients with a female phenotype, and oestrogen substitution should be initiated when puberty is anticipated (Meschede et al., 1997). Patients with a predominantly feminine phenotype often consult their physicians because of amenorrhoea or hirsutism. Male patients are usually not seen in infertility clinics.

Disorders of androgen action at target organs: androgen resistance

Androgens fail to exert their effects when the intracellular androgen receptor is functionally absent or impaired. Mutations in the androgen receptor have been localized to the X chromosome (Figure 2 and Table IV), Xq11–12, and are estimated to occur at a frequency of one per 50 000 births (McPhaul et al., 1991, 1993).

Over 200 different mutations in the gene encoding for the androgen receptor have been identified (Gottlieb et al., 1998). The relatively large number of mutations accounts for multiple syndromes of androgen resistance that are typified by qualitative or quantitative reductions in ligand binding and receptor protein, explaining the absence of ligand binding and hormone-binding site and the DNA-binding domain of the receptor protein, explaining the absence of ligand binding and qualitative or quantitative reductions in lidand binding (Marcelli et al., 1992; Quigley et al., 1995). The grades of androgen resistance find their expression in multiple syndromes characterized by different phenotypes (Table IV).

The undervirilized fertile male syndrome represents the mildest form of androgen insensitivity. Patients are not well characterized since the phenotype is normal and the incidence among infertile patients is rare (Meschede et al., 1997). Spermatogenesis may be reduced among patients with this syndrome, but fertility is unaffected (Meschede et al., 1997). The typical cellular defect in spermatogenesis is unknown since systematic studies are lacking.

The Reifenstein syndrome represents a moderate type of androgen resistance. Defects in male genitalia such as hypo- spadias and cryptorchidism are frequently evident. Patients with Reifenstein syndrome have impaired spermatogenesis and may consult their physicians because of infertility. For mild forms of androgen resistance, infertility therapy relies on assisted reproductive technology for those cases where spermatozoa are recoverable from the ejaculate or testicular biopsy. Genetic counselling is recommended in these cases.

Testicular feminization represents the most severe form of androgen resistance. Patients have a normal male karyotype of 46, XY but have phenotypic sexual features of females, including female body proportions and pseudovaginia; however, ovaries are absent, but inguinal testicles are present. The seminiferous epithelium is limited to Sertoli cells (Table IV). Testes should be removed surgically to reduce the risk of testicular cancer.

Defects in peptide hormone response elements

Spermatogenesis is supported by FSH which binds to Sertoli-cell receptors to trigger cAMP-dependent pathways, including nuclear transcription factors such as CAMP-responsive element-binding protein (CREB) and the CAMP-responsive element modulator (CREM). CREM and CREB ultimately influence the expression of genes involved in spermatogenesis such as protamine 1 and 2, transition protein 1 and 2, and others (Peri et al., 1998). Different CREM-dependent genes appear to regulate the formation of CREM repressors and activators acting at different points in the meiotic cycle (Weinbauer et al., 1998). Switching between the expression of genes directing CREM repressors and CREM activators, during the early steps of spermatogenesis, appears to be a decisive regulatory event in completing spermatid development since CREM activator expression is missing in certain severely oligozoospermic men (Peri et al., 1998; Weinbauer et al., 1998). The genes responsible for the failure in the CREM activator switch remains to be identified and these preliminary findings with oligozoospermic patients must be extended to larger groups of infertile men.

Conclusions

Widespread use of ICSI (Palermo et al., 1993) protocols raises medical concerns about the transfer of genetic defects to future generations since men with severe disorders of spermatogenesis also manifest genetic errors as part of the underlying aetiology of their defect (de Kretser, 1995; Engel et al., 1996; Tuerlings et al., 1998a). Indeed, recent investigations confirm a higher incidence of chromosomal abnormalities, such as unbalanced complements of chromosomes and delays in first-year developmental scores, in children conceived via the use of ICSI than in those conceived naturally or with other assisted reproductive procedures (Andrews et al., 1998; Bonduelle et al., 1998a,b; Bowen et al., 1998). However, most men seen in infertility clinics are free of genetic alterations that are known to cause severe somatic defects, so the transfer of defective genes to future generations via repro-
ductive technologies is unlikely to differ from that of normal fertile men. Severe chromosomal defects, such as polyplody or unbalanced translocations, usually result in embryonic death of the offspring in the first trimester of pregnancy so inheritance of major somatic defects is not expected following use of assisted reproductive techniques (Engel et al., 1996).

The percentage of infertile males with severe disorders in spermatogenesis associated with microdeletions on the Y chromosome can be expected to increase if the 10% of infertile men with such deletions rely on ICSI. Microdeletions on the Y chromosome are passed to 100% of the sons if spermatooza from patients with microdeletions are used in assisted reproduction (de Kretser, 1995; Engel et al., 1996). Infertility in this cohort of men can be treated with assisted reproductive technologies; however, such protocols pose a dilemma for future male offspring who seek treatments similar to those experienced by their fathers (de Kretser, 1995). Patients should be informed of the risk of infertility in their male offspring. Genetic counselling is recommended in all cases where chromosomal abnormalities are linked to disorders of spermatogenesis (Meschede et al., 1998).

New opportunities exist to access the genetic makeup of all cells, including individual spermatozoa by molecular analyses. Adoption of these new methods expands the opportunity to diagnose former idiopathic causes of infertility and, most importantly, to advise patients about the potential for passing on inheritable defects via the paternal genome. Current reliance on sperm karyotypes and fluorescence in-situ hybridization of sex chromosomes offers a superficial estimate of the risk involved for patients with numerical chromosomal aberrations, especially when spermatozoa are recovered from individuals with Klínefelter’s syndrome. Providing patients with access to contemporary diagnostic protocols will expand the range of treatments that can be offered to assist the infertile male as well as providing advice on the risks of transmitting abnormal genes to future children.

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